BIOGEOCHEMISTRY UNDER ICE

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By

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Abstract

Many lakes, ponds and reservoirs are subject to long and changing periods of ice cover. However, limited winter research has created key knowledge gaps. How does biogeochemical cycling change under ice? Are environmental variables and nutrient changes synchronous or asynchronous? Is winter a time of active nitrogen cycling? I undertook an intensive field campaign, studying prairie potholes ponds, reservoirs and lakes through winter, spring melt, and open water to understand the biogeochemical transformations that control nutrient flux and nitrous oxide (N_2O) production in these systems. Surprisingly, despite lower winter temperatures, winter and summer rates of denitrification did not differ. Again, despite cold temperatures, ecologically important rates of pelagic nitrification can occur and impact NH_4^+, NO_3^- and oxygen concentrations. My work in wetland ponds in winter suggested there are two phases to winter ice cover. First, I observed declining oxygen concentrations that corresponded with the accumulation of NH_4^+, soluble reactive phosphorus (SRP) and the decline of NO_3^- concentrations during winter. During this period, N_2O tended to be supersaturated. Melt conditions caused nutrient concentrations to decline, despite nutrient-rich melt water inputs. This was likely a result of increased nutrient uptake (for example, NH_4^+ uptake during melt was 50 times higher than winter uptake), adsorption and sedimentation. In a data rich reservoir (with nearly 40 years of semi-weekly physical, chemical and biological data), I identified two key phases of winter. Early to mid-winter conditions are characterized by decreasing oxygen, and increasing conductivity, NH_4^+ and SRP. Late winter conditions were characterized by increases in oxygen, corresponding to increases in chlorophyll and diatoms likely contributing to the nutrient drawdown. Together, this

work supports the narrative that small ponds, lakes and reservoirs can act as hotspots for nutrient transformations despite their small size, and suggests the timing of key chemical and biological changes are driven by changes in the physical environment. Biogeochemical processes continue through winter, despite low light and temperatures. With declining ice cover, important seasonal changes are expected, although improved light during the melt period appears to be a key moment, where chemical conditions are re-set at the onset of the open water season.

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Dedication

This thesis is dedicated to my partner in life, Zachary Keesey for his un-ending patience and support when I needed it most, to my grandmother, Gayla Colleen Cavaliere, for her belief in me, and to my mother, Gayla Maria Cavaliere for her love and support.

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List of Abbreviations

Abbreviation	Definition	Abbreviation	Definition	Abbreviation	Definition
AIC	Akaike's information criterion	Q10	factor by which rates change over a 10°C change in temperature	NO_2	nitrite ion
NH_4^+	ammonium ion	FID	flame ionization detector	N_2	nitrogen gas
NH_4-N	ammonium as nitrogen (-N)	GAM	generalized additive model	N_2O	nitrous oxide
anammox	anaerobic ammonium oxidation	GLM	generalized linear model	p	p-value
ANOVA	analysis of variance	HCl	hydrochloric acid	lmp	permutations linear model
BPWTP	Buffalo Pound Water Treatment Plant	IISD-ELA	International Institute for Sustainable Development-Experimental Lakes Area	$KHSO_4$	potassium bisulfate
CO_2	carbon dioxide	^{15}N	isotope of nitrogen	PCA	Principal component analysis
DF	degrees of freedom	LOQ	Limit of quantitation	R^2	R squared; coefficient of determination
DNRA	dissimilatory nitrate reduction to ammonium	lm	linear model	REML	restricted maximum likelihood
DIN	dissolved inorganic nitrogen	MIMS	membrane inlet mass spectrometry	rpm	rotations per minute
DOC	dissolved organic carbon	CH_4	methane	SK	Saskatchewan
DOM	dissolved organic matter	MDL	Method detection limit	SWI	Sediment water interface
ECD	electron capture detector	$\mu\Sigma$	micro Siemens	Na^+	sodium
EDF	estimated degrees of freedom	NO_3^-	nitrate ion	SRP	soluble reactive phosphorus
ELA	Experimental Lakes Area	NO_3-N	nitrate as nitrogen (-N)	SDNWA	St. Denis National Wildlife Area
				SO_4^{2-}	sulfate

1.0 INTRODUCTION TO THE DISSERTATION

1.1 Introduction

Ponds and shallow lakes can undergo the most rapid changes in winter, for example, experiencing frequent and sometimes long-term winter anoxia which affects nutrient cycling (Devito and Dillon 1993; Meding and Jackson 2003). The large area of sediment relative to overlying water means shallow lakes tend to cycle nutrients more quickly than deeper lakes (Mathias and Barica 1980), which further suggests they may be particularly dynamic in winter. Small water bodies can have an inordinate impact on nutrient cycling compared to larger water bodies, yet they are often excluded from global nutrient and greenhouse gas budgets (Downing 2010; Cheng and Basu 2017; Hansen et al. 2018). Dramatic decreases in ice-cover duration could change the structure and function of shallow lakes, ecosystems that know can be vulnerable to regime shifts (Scheffer et al. 1993). Yet, there are many unknowns about how changes in winter duration will affect shallow lakes and lentic water bodies as a whole. Due to their vulnerability, it is important to understand how winter conditions affect shallow lake, reservoir and pond biogeochemistry – the focus of my dissertation research.

My dissertation research focusses on changes in nutrient cycling under ice, answering questions like: How do biogeochemical cycles change under ice? Are environmental conditions and nutrients synchronous or asynchronous? Are the water column and sediments active sites for nitrogen cycling under ice cover?

My focus on nutrient cycling is motivated by the fact that globally, and within the prairies, nutrient pollution is considered the most important factor affecting freshwater ecosystems (Prairie Provinces Water Board 2016). Higher nutrient concentrations can enhance growth of phytoplankton sometimes leading to toxic cyanobacterial blooms, and contributing to increased anoxia risk (Jackson et al. 2001; Meding and Jackson 2003; Schindler 2012). These conditions suggest that changes in winter nutrient cycling might have important impacts on eutrophication risk in the open water season.

The Prairies are a region of many shallow lakes, reservoirs, and ponds, numbering in the hundreds of thousands (Mushet 2016). Many are naturally nutrient rich, but subject to increased nutrient loading, and to changes in local climate and hydrology (Barica 1987; Nachshon et al. 2013; PaiMazumder et al. 2013; Dumanski et al. 2015). Due to high nutrient loads, many Prairie lakes and reservoirs are susceptible to poor water quality, particularly under a changing climate due to increasing water demand, watershed modifications and drought (Schindler and Donahue 2006; Hassanzadeh et al. 2016).

1.2 Literature review

1.2.1 Physical and biogeochemical characteristics of ice-covered water bodies

Temperature, light, and water column mixing tend to be reduced in waterbodies with the onset of ice-cover. These three factors are the main drivers of biological and chemical change in winter (Bertilsson et al. 2013). Temperate lakes are typically isothermal at fall overturn, but as ice forms in early winter, a temperature and density gradient is slowly formed. The denser,

warmer water in the water column is further heated by the sediments (Bengtsson 2011), while the colder, less dense water stays near the surface as the ice forms (Catalan 1992). Low temperatures decrease microbial enzyme activity (Pomeroy and Wiebe 2001; Søndergaard et al. 2013). But the warmer sediments can maintain a higher metabolic activity than the surface waters and as such, often are sites of active respiration and mineralization (Catalan 1992; Thamdrup and Fleischer 1998).

Snow accumulation on the surface of ice-covered lakes, reservoirs and ponds decreases light penetration (Catalan 1992), hence snowfall, blowing snow, and snowmelt alters light inputs over winter (Catalan 1992; Pernica et al. 2017). Light penetration can heat the water below, causing mixing (Bertilsson et al. 2013; Pernica et al. 2017), and altering the light environment for phytoplankton. Typically, early winter is characterized by low light conditions associated with the accumulation of snow (Catalan 1992). Towards the end of winter, snow cover often decreases before ice is lost, leading to major increases in light penetration into the water column. The type of ice also alters the light environment, with more opaque, or white ice blocking much of the light compared to black or crystal ice that is more transparent (Schindler et al. 1974; Petrov et al. 2005).

Mixing in ice-covered lakes, reservoirs and ponds is limited, due to isolation from wind-action, low hydrologic inputs and low light (Bengtsson 1996). Despite this, there are certain conditions that can induce some winter mixing. Light, as mentioned, can induce mixing if light penetration is sufficient to heat the water under the ice, inducing convective mixing as the denser, warmer water sinks (Pernica et al. 2017). Heat from the sediments can also warm lake water, and stimulate convective mixing (Bengtsson 1996). Water moving into the lakes under ice cover from a river or ground water inputs can cause mixing, however, this mixing can vary depending on the

size and duration of water inputs (Bengtsson 1996). Although the impact of wind is significantly dampened as compared to the open water season, wind can still influence lake mixing as it pushes against the sheets of ice, causing tilting and oscillations which drive small circular movements in the water under the ice (Bengtsson 1996).

Prairie water bodies are often naturally ion rich and show a wide range of salinities (D et al. 2009; Pham et al. 2009). The exclusion of salts during ice formation increases lake salinity over winter (Pieters and Lawrence 2009). Given that ice-cover can account for a large proportion of water volumes (approximately 5% to 50% of the total volume in the prairie lakes, reservoirs, and ponds of Chapters 2–5), it is clear that ion exclusion from the ice can dramatically change the winter chemistry of, in particular, shallow water bodies. Increased salinity over winter can influence biological and chemical processes, including enhanced P release (Caraco et al. 1993), and has a strong influence on mixing patterns. For example, a salinity gradient can form as fresh melt water sits on the more saline lake water (Bergmann and Welch 1985).

Ice-covered water bodies are isolated from atmospheric gas exchange (Meding and Jackson 2003; Denfeld et al. 2018). Due to this isolation, photosynthesis (when light is sufficient) is typically the only source of oxygen production. Yet the sinks of oxygen are plentiful in winter and include organic matter mineralization, nitrification and methanotrophy (Knowles and Lean 1987; Baehr and DeGrandpre 2002; Denfeld et al. 2018). Rates of oxygen depletion in lakes are strongly related to depth (Meding and Jackson 2003). As a result, shallow lakes are frequently subject to anoxia (Meding and Jackson 2001) due to the proportionally greater sediment surface area compared to deeper lakes (Mathias and Barica 1980). Shallow water bodies are also more likely to produce higher concentrations of greenhouse gases – carbon dioxide (CO_2) and methane (CH_4) – than deeper lakes due to their higher organic matter content (Denfeld et al. 2018).

Sediment and water temperature can influence rates of oxygen decline. Warmer sediments and water will increase rates of bacterial respiration and chemical oxidation reactions (Golosov et al. 2007; Terzhevik et al. 2009). This too can influence seasonality of key biogeochemical processes. During the fall, the deposition of organic matter, combined with relatively warm bottom sediments can increase the rate of oxygen depletion at the onset of ice-cover (Terzhevik et al. 2009).

1.2.2 Nutrient cycling in winter

Lakes receive nutrients from a variety of external sources, including atmospheric deposition, runoff, local terrestrial inputs and groundwater (Bertilsson et al. 2013). In ice-covered lakes, the external nutrient supply is limited due to a decline in most of those sources (Bertilsson et al. 2013), with groundwater as the only hydrologic input likely to continue with similar rates of input across seasons. Surface water inputs are typically low, particularly in regions such as the Canadian prairies, where spring snowmelt dominates runoff. Under ice-cover, nutrient dynamics reflect a balance between algal and microbial uptake, remineralization and sediment release (Agbeti and Smol 1995; Bertilsson et al. 2013), with the balance of these processes expected to vary over time due to the changing chemical and physical conditions discussed previously.

Oxygen concentrations influence both nitrogen (N) and phosphorus (P) dynamics. Nitrification and P sorption can occur under aerobic conditions, changing the nutrients available for biological activity (Pauer and Auer 2000; Kleeberg et al. 2013), while anaerobic conditions can stimulate N removal via denitrification and P release (Knowles 1982; Nürnberg 1984). Recent work in winter has revealed that N has a tendency to accumulate over winter (Hampton et al. 2017; Powers et al. 2017b; Cavaliere and Baulch 2018). Specifically, nitrate (NO_3^-) can

accumulate in the presence of oxygen – likely as a result of nitrification (Powers et al. 2017a; b), and high rates of winter NO_3^- accumulation through nitrification have been associated with oxygen decline (Chapter 3; Knowles and Lean 1987; Powers et al. 2017a). Winter NH_4^+ concentrations have been correlated with summer algal blooms (Barica 1975; Murphy and Brownlee 1981), suggesting a potentially important link between winter and summer conditions. Although enhanced P release from sediments under anoxic conditions is common (however, not universal), high rates of winter P release may be rare (Boström et al. 1988; D'Silva 2017; Orihel et al. 2017).

For much of the winter, phytoplankton growth is slow (Catalan 1992; Gu and Alexander 1993; Katz et al. 2015). However, under little snow, higher light conditions, photosynthesis and autotrophic growth can be substantial (Catalan 1992; Bertilsson et al. 2013; Pernica et al. 2017). The oxygen produced tends to be concentrated near the ice-water interface where algae often congregate due to higher light availability (Catalan 1992; Gu and Alexander 1993; Bertilsson et al. 2013). However, shallow mixing can affect the environment experienced by phytoplankton, creating a highly dynamic light environment and moving oxygen within the water column (Pernica et al. 2017). Nutrients are taken up as phytoplankton photosynthesize and can decrease nutrient concentrations near the ice (Bertilsson et al. 2013).

Winter N cycling will be affected by changes in N inputs, changes in the physical and chemical environment, and changes in biological activity (Knowles and Lean 1987; Catalan 1992; Hampton et al. 2017; Powers et al. 2017a). Changes in oxygen availability and temperature are critical controls on rates of nitrification and denitrification. Denitrification is the microbial reduction of nitrogen oxides (NO_3^- and NO_2) to nitrogen gases (NO, N_2O, and N_2; Knowles 1982) which occurs under anoxic conditions through the following sequence:

$NO_3^- \Rightarrow NO_2^- \Rightarrow NO \Rightarrow N_2O \Rightarrow N_2$

The process is typically coupled to the consumption of an organic carbon substrate, with an example of the stoichiometry of the reaction shown here for glucose consumption and reduction of NO_3^-, from Wetzel (2001 p. 217):

$C_6H_{12}O_6 + 12\ NO_3^- \Rightarrow 12NO_2^- + 6CO_2 + 6H_2O$

And nitrite reduction:

$C_6H_{12}O_6 + 8NO_2^- \Rightarrow 4N_2 + 2CO_2 + 4CO_3^{2-} + 6H_2O$

Denitrification is an important process for removing NO_3^-. Rates of denitrification are controlled by concentrations of NO_3^-, oxygen and organic matter, and by temperature (Knowles 1982; Seitzinger et al. 2006). Denitrification occurs in anoxic zones that are connected to a source of NO_3^- (Seitzinger et al. 2006). Cooler temperatures decrease denitrification rates, and can yield higher production of the intermediaries (NO and N_2O; e.g., at temperatures between 0 and 5°C; Knowles 1982).

Nitrification during winter affects lake NH_4^+ and NO_3^- concentrations (Knowles and Lean 1987; Powers et al. 2017a), as well as concentrations of O_2. Nitrification is performed by microbes through the oxidation of NH_4^+ to NO_3^- by a two-step process (Pauer and Auer 2000), as per Wetzel (2001), first ammonium is converted to nitrite:

$NH_4^+ + 1½\ O_2 \Rightarrow 2H^+ + NO_2^- + H_2O$

Then nitrite is converted to nitrate:

$NO_2^- + ½O_2 \Rightarrow NO_3^-$

Intermediates in the nitrification process include nitrous and nitric oxides, and NO_2^- (Laanbrock and Bollman 2011). Rates of nitrification are controlled by temperature, pH, oxygen and NH_4^+ concentrations (Knowles and Lean 1987; Wetzel 2001; Powers et al. 2017a). Although

low temperatures may inhibit nitrification (Pauer and Auer 2000), the process has been observed at temperatures as low as 2-3°C, despite the presence of very low concentrations of nitrifying bacteria (Knowles and Lean 1987).

Nitrification in winter is understudied, but is potentially important to the oxygen dynamics of ice-covered lakes. For example, in a mesotrophic lake in Ontario, the water column oxygen consumption due to nitrification accounted for 71% of the total winter oxygen depletion (Knowles and Lean 1987). Temperate lakes in Wisconsin, however, exhibited a lower range of nitrification oxygen demand (1-25% of observed oxygen decline; Powers et al. 2017a).

The winter nitrogen cycle can also be important in greenhouse gas accumulations under ice cover because of periodic winter anoxia, organic carbon accumulation and isolation from the atmosphere fostering gas accumulation (Soued et al. 2015; Denfeld et al. 2018). Nitrous oxide (N_2O) is one such greenhouse gas and is produced by the processes of nitrification and denitrification (Firestone and Davidson 1989). This gas is also a driver of stratospheric ozone depletion (Ravishankara et al. 2009). Water bodies can act as sources or sinks of N_2O often controlled by temperature, NO_3^- and NH_4^+ availability, organic matter and possibly even sulfate (Baulch et al. 2011; Soued et al. 2015). These factors are commonly considered primary controls on both nitrification and denitrification and as such are controls on N_2O production (Knowles 1982; Thamdrup and Fleischer 1998; Seitzinger et al. 2006; Souza et al. 2014). However, the yields of N_2O from each process (nitrification: N_2O: NO_3^- and denitrification: N_2O:N_2) are also variable – making prediction of N_2O emissions particularly challenging. N_2O yields are controlled by a similar suite of environmental conditions to the rates of nitrification and denitrification, including substrate availability, temperature, pH and oxygen concentrations (Firestone and Davidson 1989; Baulch et al. 2011).

Overall, N_2O dynamics in ice-covered lakes are poorly studied, outside of the boreal region (Soued et al. 2015). Work in boreal reservoirs reveals that the ice-cover period can be an important period of N_2O production and the outgassing during spring can be substantial (as much as 15% of the annual N_2O budget; Soued et al. 2015). Nitrate concentrations are positively related to production of N_2O in winter in both boreal lakes and more southerly streams (Baulch et al. 2011; Soued et al. 2015), suggesting changes in substrate availability in winter could impact N_2O production. In winter, although low temperatures will reduce metabolic activity of microbes, increased N_2O production may occur (Baulch et al. 2011; Soued et al. 2015) and due to limited gas exchange will accumulate over winter. However, it appears that aquatic ecosystems can also function as N_2O sinks under these cold winter conditions, when oxygen concentrations are low, and ample organic matter is available (Soued et al. 2015). Ultimately, more work is needed to establish controls on the winter sources or sinks of N_2O, under the range of environmental conditions observed. For example, work in a shallow ice-covered eutrophic lake demonstrated higher N_2O concentrations were associated with low oxygen and low NO_3^- concentrations (Knowles et al. 1981) suggesting important interactions between nitrogen, oxygen, and organic carbon availability may mediate differing patterns of N_2O production and consumption through winter.

Nitrogen transformations can also include anammox (anaerobic NH_4^+ oxidation which produces nitrogen gas while also consuming nitrite), DNRA (dissimilatory NO_3^- reduction to NH_4^+; Burgin and Hamilton 2007) and N fixation (fixing N_2 to biologically available N; Fowler et al. 2013). These processes can be important to the N cycle. Nitrogen fixation is thought to be restricted to warmer periods (Goering and Neess 1964), while anammox and DNRA remain understudied within freshwaters but are likely important in anaerobic environments where NH_4^+

is available in conjunction with nitrite (anammox) or when NO_3^- is available and can be converted to NH_4^+ under conditions favorable for fermentation or sulfur oxidation (DNRA; Burgin and Hamilton 2007).

Mineralization of organic matter releases dissolved N and P. Shallow lakes, due to their large surface area to volume ratio, are impacted by sediment mineralization more than deeper lakes (Scheffer et al. 1993). Release of nutrients by mineralization of organic matter is likely important in winter and can be controlled by low winter temperatures and by the availability and stoichiometry of substrates (Gudasz et al. 2015). In winter, due to lower temperatures and metabolic activity, mineralization of organic matter could decrease (Thamdrup and Fleischer 1998). However, there is evidence that nutrients tend to accumulate over winter (Catalan 1992; Hampton et al. 2016; Powers et al. 2017b) and in shallow lakes, sediment mineralization is likely a key process driving this accumulation combined with lower autotrophic demand for nutrients (Gu and Alexander 1993; Joung et al. 2017).

The phosphorus cycle in lakes has fewer processes than the nitrogen cycle, but is similarly complex, in part due to the wide range of controls on phosphorus storage or release from sediments. Sediments can be a source or sink of P in lakes (Boström et al. 1988). Several factors determine whether P is going to be released or retained by sediments, including planktonic activity, rates of sedimentation, and the chemistry of the sediment water interface (SWI; Orihel et al. 2017), all of which may be impacted by winter conditions.

Oxygenated water at the SWI can prevent release of iron-bound P but during periods of low oxygen, particularly at the SWI, iron-bound P can be mobilized into the overlying water (Kleeberg et al. 2013). Given winter can be a time of low oxygen concentrations for shallow lakes, where redox-sensitive phosphorus binding is important, phosphorus release from sediments

seems likely (Meding and Jackson 2003). Availability of these iron oxy-hydroxides is not the only factor controlling P binding. The presence of sulfate can also change the iron-P binding relationship (Orihel et al. 2017). This is particularly important in these prairie study sites, as sulfate is abundant in this area and ion exclusion will increase sulfate concentrations through winter (Dugan et al. 2017; Orihel et al. 2017). Sulfate can compete for binding sites with iron in sediments, and, under anoxic conditions, FeS as the sulfate is further reduced after the iron is reduced to iron(II) (Orihel et al. 2017). Given what we know about prairie waterbodies, it is likely that the formation of FeS is common, particularly in winter (Orihel et al. 2017). The presence of nitrate in the system can inhibit P release under anoxic conditions because before iron oxy-hydroxides are reduced and P is released, nitrate has to be reduced (Orihel et al. 2017). Further, nitrate reduction (denitrification) could labile organic matter, inhibiting further reduction processes including reduction of iron oxy-hydroxides (Orihel et al. 2017).

Despite the prevailing classical model of P release from the coupled cycling of iron and P under anaerobic conditions (Nürnberg 1984), recent work has shown that oxic release of P can be quite important (Gächter and Müller 2003; Orihel et al. 2017). Oxic release of P is worth considering in winter as an important P release mechanism because not all lakes experience anoxia in winter, even at the SWI. Further, enhanced P release could occur as sedimentation rates can be particularly high in winter (Catalan 1992) and oxygen presence could increase rates of organic matter mineralization releasing organic P or inorganic P (Orihel et al. 2017; Shinohara et al. 2018).

Changes in pH over winter can alter P chemistry in sediments. Increased concentrations of carbon dioxide as the water body is isolated from the atmosphere can decrease pH through production of carbonic acid (Wetzel 2001). The lower pH could increase dissolution of the P

containing calcite mineral, while, less likely in winter, higher pH will also cause P release through higher affinity for hydroxyl ions to replace more mobile P ions (Orihel et al. 2017). Salts and other ions tend to increase in winter due to their exclusion as the ice forms (Pieters and Lawrence 2009) and could increase P release from sediments due to competition for binding sites with ions (Caraco et al. 1990; Spiteri et al. 2008; Orihel et al. 2017).

Nutrient inputs in winter may further impact the P cycle. For example, elevated organic P inputs relative to inorganic P may occur in winter and spring (Shinohara et al. 2018). Organic P could be an important and labile source of P to winter plankton communities (Orihel et al. 2017; Shinohara et al. 2018). Use of available P by plankton communities composed of both autotrophs and heterotrophs could also present a temporary sink for P. The growth and death of plankton biomass and subsequent sedimentation and burial could remove labile P from the system (Catalan 1992; Wetzel 1992). In winter, the likelihood of sedimentation of P-rich biomass could be enhanced due to limited mixing, although limited by new biomass production.

Overall, P release is likely in winter in the prairies. The prairie waterbodies are dominated by high trophic statuses (eutrophic and hypereutrophic) which are prone to P release (North et al. 2015; Doig et al. 2017; Orihel et al. 2017), and these water bodies have a tendency to go anoxic in winter and sulfate concentrations are high (Barica and Mathias 1979; Meding and Jackson 2003; Orihel et al. 2017).

1.2.3 Spring melt

Spring melt brings dramatic shifts in oxygen, light and temperature. These changes can occur at a particularly sensitive time in terms of the connectivity of ecosystems. Within the prairies, spring melt is the period of maximum potential connectivity of seasonally isolated wetlands. That

is, the large quantities of water that move during snowmelt can induce filling and spilling of wetlands, facilitating nonlinear changes in flows, and loads to downstream ecosystems (Phillips et al. 2011; Shaw et al. 2012). It seems this period of maximum hydrologic connectivity can co-occur with the period where risk of high nutrient concentrations is greatest (Petrone et al. 2007; Cade-Menun et al. 2013; Costa et al. 2017). This suggests that winter and spring conditions within ponds may be important determinants of downstream solute export (Leibowitz and Vining 2003; Hayashi et al. 2016).

Spring melt is a highly dynamic period, and the exact timing of changes is critical. The confluence of increased light and winter accumulated nutrients can often lead to a dramatic spring bloom. The magnitude of this bloom can be important to annual productivity, for example, the spring bloom reaches a biomass 1.5 to 6-fold higher than the summer biomass in Lake Erie (Reavie et al. 2016). Oxygen is reinjected into anoxic systems, and also tends to increase in oxic systems with the increased productivity, and gaps in the ice cover (Catalan 1992). The timing of spring melt, and the spring bloom are changing (Winder and Schindler 2004), and decreasing ice-cover duration and warming temperatures are likely to result in shifts in the spring bloom community (Horn et al. 2011). This spring melt period is expected to be a highly dynamic time for biogeochemical cycling with the influx of nutrients from the catchment, changes in oxygen, light, mixing, and primary productivity. As such, snowmelt and ice-out are expected to be complex and interesting 'hot moments' where hydrologic changes and biogeochemical changes co-occur.

1.3 Dissertation chapters

This dissertation has four manuscript-style chapters which are centered on the question of how lakes, ponds and reservoirs change through winter months. Chapters 2 and 3 assess rates of denitrification and nitrification in ice-covered lakes, respectively. The processes of nitrification and denitrification are important because high nitrogen concentrations can negatively impact water quality, supporting excessive growth of algae in conjunction with sufficient sources of phosphorus and light (Schindler and Fee 1974; Conley et al. 2009). Nitrification does not directly affect concentrations of dissolved inorganic nitrogen, but instead, affects the speciation of dissolved inorganic nitrogen, transforming it from the reduced form of ammonia, to NO_3^-, with this speciation of nitrogen also impacting algal communities (Glibert et al. 2016; Glibert 2017). Nitrification can also be an important oxygen sink in some ecosystems in winter (Knowles and Lean 1987; Powers et al. 2017a), although this process has received surprisingly little study in freshwaters in any season. By producing NO_3^-, nitrification yields the substrate for denitrification, a permanent removal pathway for fixed nitrogen, which is recognized to be an important nitrogen loss pathway in lakes (Seitzinger 1988), but has not been directly measured under ice cover. Through experimental determination of rates of nitrification and rates of denitrification, assessment of controls on these processes, and measurement of the greenhouse gas N_2O which is produced via nitrification and denitrification, Chapters 2 & 3 provide first insights into understanding these two key nitrogen cycling processes within lakes under ice cover and implications to our understanding of N_2O budgets.

The fourth chapter focuses on how prairie pothole ponds change through winter, and through the dynamic period of ice melt. Often difficult to study, the melt period is largely overlooked in current literature (but see Denfeld et al. 2015, 2016b; Canelhas et al. 2016). It is a period of

gradually, sometimes rapidly, depleting ice-cover, during which the lentic system develops a different physical regime. The melting ice and overlying snow allow increased light input and introduces snow and melt water to the lake. Gas exchange resumes, as lakes and ponds develop ice-free areas, first typically around the edges. Mixing dynamics are also altered by changes in ice, and in the light environment. This chapter explores winter trends of conductivity and nutrients, and the changes observed as winter transitions to the spring-melt period. The fifth chapter uses a long-term water quality data set to answer questions about oxygen, nutrient and phytoplankton changes during ice-cover. This analysis gives us an over-arching understanding of nutrient dynamics under ice, a complex period of multi-directional change. Together, these contributions provide insights ranging from understanding of changes within individual biogeochemical processes, to a whole ecosystem view of changing chemistry through winter. The overarching aim of this dissertation is to inform our understanding of lakes, ponds and reservoirs during ice cover, focusing on understanding changes in nutrient concentrations, and nutrient cycling. Winter remains understudied (Salonen et al. 2009; Hampton et al. 2015), yet is a period that is rapidly changing, with declining periods of ice cover (Lemke et al. 2007; Bertilsson et al. 2013). Ultimately, a better understanding of conditions during winter is required to help anticipate how these ecosystems may change as a result of declining ice cover.

2.0 DENITRIFICATION UNDER LAKE ICE

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2.1Abstract

Many lakes, ponds and reservoirs are subject to long and changing periods of ice cover. However, limited winter research has created key knowledge gaps. How does nitrogen cycling change under ice? And what does changing ice cover duration mean for water quality? Here we present the first measurements of denitrification rates under ice in temperate, polymictic water bodies. Surprisingly, despite lower winter temperatures, winter and summer rates of denitrification did not differ. Experimental work suggests that denitrification rates are controlled hierarchically, first by NO_3^- concentrations, then by temperature. As a result, controls on NO_3^- inputs such as changing hydrology and nitrification, combined with physical controls on delivery of NO_3^- to sediments, may be more important to NO_3^- retention via denitrification than the duration of low temperature or ice cover. Nitrous oxide was typically supersaturated under-ice, suggesting an ice-out flux will occur, and this flux may be greatest in systems with elevated NO_3^- .

2.2 Introduction

Sustained, high fluxes of nitrogen (N) into aquatic ecosystems continue to occur across most of the globe (Causse et al. 2015) causing myriad environmental effects in freshwaters, and downstream coastal waters. A key partial counterbalance to these increased N inputs is denitrification, the anaerobic reduction of nitrate (NO_3^-) to N_2 gases (Knowles 1982; Seitzinger et al. 2006). Freshwater bodies play an important role in removing fixed N from the bioavailable pool; with denitrification in lakes, rivers and reservoirs accounting for as much as 20% of total global N removal (Seitzinger et al. 2006; Harrison et al. 2009). Our current understanding of the nitrogen cycle is largely limited to the open water season. However, long periods of ice cover are common at northern latitudes. This has resulted in major gaps in our understanding. Given evidence of rapid ice cover decline in many regions, and predicted further changes to ice cover duration and thickness (Lemke et al. 2007; Bertilsson et al. 2013), we may anticipate changes in nitrogen cycling as winter conditions recede and make way for a longer open water period.

The process of denitrification is controlled by oxygen and NO_3^- concentrations, temperature and availability of organic matter (Knowles 1982; Seitzinger et al. 2006). To date, there have been few measurements of denitrification rates in ice covered temperate water bodies (Myrstener et al. 2016); however, work in cold, but ice-free, lakes suggests mixed impacts of season on denitrification rates (Martin et al. 2001; Appendix A Table A.3), and work in subarctic, boreal, and Antarctic lakes demonstrates that denitrification is active when environmental conditions are suitable despite cold temperatures (Goering and Dugdale 1966; Ward et al. 2005; Myrstener et al. 2016).

Other winter changes can also be important to denitrification, including changes in nutrients and oxygen (Catalan 1992; Bertilsson et al. 2013). Nutrient accumulation may be favored through winter – with continued mineralization of organic matter but low algal uptake (Catalan 1992; Hampton et al. 2017). For example, a recent 101-lake assessment showed higher total nitrogen in winter, under ice-cover, than in summer (Hampton et al. 2017). While this could indicate the potential for enhanced substrate availability, changes in NO_3^- availability through winter are not well characterized (Catalan 1992). Nitrate availability can be a dominant control on denitrification (Brin et al. 2017). As a result, autumn inflows of NO_3^- or production of NO_3^- through nitrification in winter (Powers et al. 2017a) may be important determinants of NO_3^- removal via denitrification in winter. Low oxygen conditions are common in winter (Barica 1987), and may support increased rates of denitrification (Seitzinger et al. 2006), however, this can also lead to greater spatial separation between zones of nitrification and denitrification. Typically, declines in oxygen are observed through winter, driven by continued respiration, and low rates of oxygen replenishment (Hampton et al. 2017). This is due to the absence of air-water gas exchange through ice, and the importance of snow and ice in limiting light and photosynthetic oxygen production (Bertilsson et al. 2013; Pernica et al. 2017). Dissolved organic matter (DOM) quantity and quality can also change through winter, particularly in small water bodies (Hampton et al. 2017), and where DOM limits denitrification (e.g., see Harrison and Matson 2003), this too could affect denitrification rates. Winter DOM changes may be driven by processes including DOM exclusion from the ice, changing photochemistry, and release from the sediments (Belzile et al. 2002), although relatively little is known regarding how DOM changes through winter, and how these changes may differ across lakes (Bertilsson et al. 2013; Hampton et al. 2017).

Changes in nitrogen cycling also have the potential to impact production and emissions of N_2O. Nitrous oxide in the troposphere acts as a potent greenhouse gas. In the stratosphere, N_2O is responsible for ozone depletion – and is currently the single most important ozone depleting gas (Ravishankara et al. 2009). Nitrous oxide is produced as a result of nitrification and denitrification (Firestone and Davidson 1989). Increased nitrogen inputs into surface waters have led to elevated emissions of this greenhouse gas from inland waters (Seitzinger et al. 2006; Baulch et al. 2011). Emissions are governed both by rates of nitrogen cycling, and the yield of these processes (N_2O: NO_3^- for nitrification, N_2O:N_2 for denitrification), with evidence that the yields can be highly variable, and influenced by N substrate concentrations, as well as other conditions (Baulch et al. 2011; Soued et al. 2015) – including pH and oxygen, which show significant winter-summer variation (Bertilsson et al. 2013). The cessation of gas exchange in winter when there is complete ice cover means N_2O produced in winters is retained in lakes, with a potential pulse of emissions occurring at ice-out (Soued et al. 2015).

Enhancing knowledge of the winter nitrogen cycle is important to understanding future changes in lake, reservoir, and pond chemistry. Yet little is known about winter. In this study we assess 3 questions: 1) Does denitrification vary between winter and summer periods? 2) Does temperature or NO_3^- control rates of denitrification? And 3) Is the accumulation of N_2O related to rates of denitrification, or NO_3^- supply? To answer these questions, we sampled 9 polymictic water bodies and experimentally determined denitrification rates under variable temperature and NO_3^- conditions. These ecosystems have large seasonal temperature changes in the sediments, and are subject to significant interannual variation in ice cover duration.

2.3 Methods

2.3.1 Study sites and sampling

We studied a series of polymictic lakes, reservoirs and ponds in Saskatchewan, Canada, with a broad range of dissolved nitrogen concentrations (Appendix A Table A.1). The primary ecosystem services provided by these systems vary and include drinking water supply, recreational waters, and important wildlife habitat (Allan and Roy 1980; Pomeroy et al. 2005). Given relative water scarcity in this semi-arid region, degraded water quality is a particularly serious concern (Barica 1987; Schindler and Donahue 2006). These systems are in agriculturally impacted catchments and some (St. Denis Pond 1, St. Brieux and Lenore Lake) have elevated conductivity (up to 5072 μS cm^{-1}), consistent with the semi-arid climate of the region and internal drainage. They are productive (mesotrophic to eutrophic; Allan and Roy 1980) and given their often shallow depths (average depth ranged from 1.3 to 14.3 m, with an average of 6.5 m; Appendix A Table A.1), they experience frequent mixing during the summer. This results in warmer sediment temperatures in the summer and cooler temperatures in winter. Several are subject to long periods of winter anoxia. At the time of winter sampling, six out of the nine study sites had oxygen concentrations at or below 2 mg L^{-1} near the sediment: Blackstrap, Broderick, Buffalo Pound, Lenore Lake, St. Brieux, and St. Denis (Appendix A Table A.1). Near sediment summer oxygen ranged from below 2 mg L^{-1} (Katepwa, St. Brieux and St. Denis) to as high as 10 mg L^{-1}, with a median oxygen concentration of 7.5 mg L^{-1}.

Winter samples were obtained by augering through ice cover, and using a heated tent to prevent freezing of water, sediments and equipment during the often extremely cold conditions of Saskatchewan winter. Summer samples were collected by boat. Sediment samples were

collected using an Ekman grab (Standard Ekman Grab, Wildco ®, Yulee, FL) in each water body in both winter and summer seasons (years 2014 – 2016) and placed into new sealable plastic bags. Samples were collected from near the center of the water body, with the exception of Katepwa Lake. Katepwa Lake is the deepest of our study systems (Appendix A Table A.1), and in this lake, samples were obtained from the nearshore at a depth of 2 m. Water samples for chemistry analyses were collected using an acid washed tube attached to a peristaltic pump (GeoPump easy-load II, GeoTech Environmental Equipment, Inc. Barrington, IL) and transferred into acid washed carboys. Water samples and N_2O samples were collected from ~50 cm above the sediment surface to avoid sediment disturbance. Nitrous oxide samples were collected by overfilling a 1.2-L bottle, and performing headspace equilibrations (Cole et al. 1994). Headspace samples were transferred to Exetainer vials with double-wadded caps. *In situ* measurements of oxygen, temperature, pH, and specific conductance were made near the sediment-water interface using the YSI 556 Multi Probe System (YSI Environmental, Yellow Springs, OH).

2.3.2 Laboratory and experimental methods

Water samples were filtered upon return to the laboratory through pre-rinsed 0.2 μm polycarbonate filters (A.M.D. Manufacturing, Mississauga, Ontario). Filtered water samples were analyzed for nitrate (NO_3^--N and NO_2-N EPA method 353.2, hereafter referred to as NO_3^-), NH_4^+ (EPA 350.1), and alkalinity (EPA method 310.2) using the SmartChem 170 autoanalyzer (Westco Scientific Instruments, Inc., Brookfield, CT). Ammonium was analyzed within 24 hours or less while sulfate and NO_3^- samples were frozen after filtering, thawed and then analyzed. Alkalinity was measured on refrigerated filtered water.

Denitrification experiments were performed using the chloramphenicol-amended acetylene block method described in Smith et al. 1978. Sediment and water samples were stored at 4ºC (<24h) and kept at 4ºC or temperature acclimated at 20ºC as per Martin et al. (2001) prior to onset of the experiment. Media bottles with septa (140 mL) were filled with sediment (25 mL) and water (50 mL) slurries from the study site and purged with nitrogen gas. Acetylene was added to experimental units to stop N_2O from being converted to nitrogen gas (Smith et al. 1978), and denitrification was measured as the accumulation of N_2O individually collected at 20, 70, 120, 170 and/or 210 minutes.

To assess temperature sensitivity of denitrification rates, we measured denitrification rates at 4ºC and 20ºC for both winter and summer obtained samples. To test for NO_3^--limitation of denitrification rates, we measured denitrification rates under ambient, and amended NO_3^- conditions (100 mg NO_3^--N kg sediment^{-1}), at both 4ºC and 20ºC and in both winter and summer seasons, as per Groffman et al. (1999). The NO_3^- addition treatment allowed us to measure denitrification rates that were not NO_3^--limited. As a result, experimentally-enriched NO_3^- concentrations were in excess of anticipated *in situ* concentrations. For each site and date, 16 replicates were employed: two controls (no acetylene), three ambient NO_3^- (with acetylene) and three NO_3^- amended (with acetylene) replicates for two temperature treatments. This design, and the inclusion of both open water and winter experiments, allowed us to test for the impacts of temperature, seasonality and NO_3^- addition on denitrification rates.

The chloramphenicol-amended acetylene block method allows rapid processing of a large number of samples using relatively low-cost techniques, but can lead to underestimation of rates in low NO_3^- systems, where coupled nitrification-denitrification is important (Smith et al. 1978; Seitzinger et al. 1993; Groffman et al. 2006). Despite these limitations, acetylene-based methods

have shown reasonable agreement with other approaches and are a practical option for experimental work which necessitates large numbers of measurements (Groffman et al. 2006).

All gas samples (from denitrification experiments and field-samples) were analyzed for N_2O on the Scion 456 Gas Chromatograph (Bruker Ltd.) using the micro-electron capture detector (ECD). Nitrous oxide concentrations were calculated using solubility equations (Weiss and Price 1980). For saline systems (St. Denis, St. Brieux Lake and Lenore Lake), we calculated ionic salinity (as per Pawlowicz 2008) due to the dominance of sulfate, magnesium, and calcium ions. These corrected salinity values were then used to determine dissolved concentrations and % saturation (Weiss and Price 1980).

2.3.3 Statistical analysis

All statistical tests were performed using R: A Language and Environment for Statistical Computing, 2016 (R Core Team 2018). Denitrification rates were determined based on cumulative N_2O production over time using the linear portion of the data. In 11% of cases this meant a time point (typically the final point) was omitted to adhere to the assumption of linearity. Individual replicates were only used if a linear relationship of cumulative N_2O to time was observed and significant, (lm, $P \leq 0.05$), indicating active denitrification, as per Groffman et al (2006). Non-linearity may reflect possible chloramphenicol interference with denitrifying enzymes or experimental error (Wu et al. 1995). In 10% of cases, a replicate bottle was removed when rates were deemed unmeasurable. To test for potential bias associated with these replicates where measurable rates could not be determined, all statistical tests were run with these samples excluded, and with rates for these bottles set to zero. Statistical analyses were robust to these changes – that is, outcome of statistical tests

was the same whether we excluded these replicates, or included them, using rates of zero. Control samples (without acetylene) exhibited linear accumulation of N_2O less than 25% of the time. The rates of N_2O accumulation in these control samples were less than 1% of denitrification rates measured in acetylene-treated vials at ambient NO_3^- concentrations. Denitrification rates were not corrected for N_2O production in controls.

Median denitrification rates were calculated from three replicates from each site and treatment. The mean coefficient of variation for replicates was 30%. The resulting data were non-normal with heteroscedastic variances, however, transformations did render them normal. Using the Box-Cox transformation for linear models, a power transformation was identified for each grouping of data, and data were subsequently transformed and tested for normality using the Shapiro Wilks Test for Normality (Venables and Ripley 2003; R Core Team 2018). Subsequently, to discern categorical differences or correlations, paired t-tests (t.test) and linear models (lm) were used (R Core Team 2018).

Data that could not be transformed to normality (nutrient chemistry, dissolved gases) were analyzed via non-parametric, permutation tests (also called randomization or re-randomization tests) (Kabacoff 2011). Permutation tests calculate a t-statistic to compare the groups of interest and then randomize the data into new groups (without consideration for the original groupings) and calculate a distribution of t-statistics for each of the possible permutations of the random groupings. If the original grouping's t-statistic falls outside of the distribution of those empirically distributed t-statistics, with a set alpha, then the null hypothesis is rejected, and the groups are deemed different (Kabacoff 2011). The package "stats" contains the Wilcoxon-Mann-Whitney signed rank test (wilcox.test) and was used to compare winter and summer physical and nutrient data (R Core Team 2018). The linear model using permutations (lmp) was used to test

for correlation between denitrification rates and NO_3^- concentrations and NO_3^- concentrations and percent saturation of N_2O (Wheeler and Torchiano 2016a)..

2.4 Results

Winter denitrification rates at ambient NO_3^- concentrations and temperatures varied from 1.5×10^{-5} to 6.7×10^{-2} μg N g^{-1} h^{-1}, while summer rates varied from 6.8×10^{-5} to 9.9×10^{-3} μg N g^{-1} h^{-1} (Fig. 2-1). There was no relationship between winter and summer rates for ambient (transformed ambient data, paired linear model, P= 0.96, 14 DF) or amended NO_3^- (transformed, paired linear model, P=0.18, 16 DF). Likewise, there was no relationship between ambient and nitrate-amended denitrification rates within seasons (winter transformed, paired, linear model P=0.69, 14 DF; or summer transformed, paired, linear model P=0.69, 16 DF, Fig. 2-2a). Nitrate concentrations did not significantly predict denitrification rates for summer or winter under seasonal temperatures and NO_3^- concentrations (linear model permutation test, P = 0.18, 6 DF). However, there was a significant relationship between winter NO_3^- concentrations and winter N_2O saturation (linear model permutation test, P = 0.03, adjusted R^2 = 0.48, 6 DF, Fig. 2-2b).

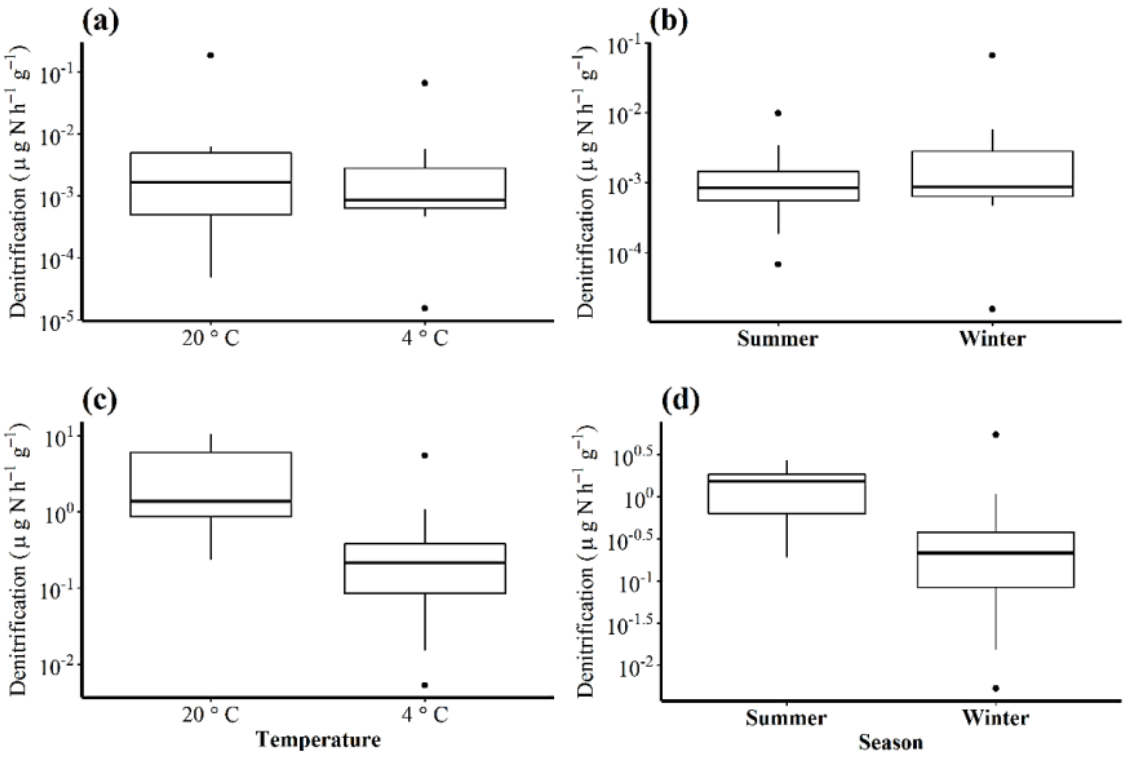

Figure 2-1 Denitrification rates across 9 lakes reservoirs, and ponds in samples obtained a) during winter and incubated at ambient and increased temperature, b) during summer and winter at ambient temperature, c) during winter at amended NO_3^- concentrations, and d) during winter and summer at amended NO_3^- concentrations. The boxplot and whiskers encompasses 95% of the data observed. Dots are outliers, inside the box are the first and third quartiles, and the center line is the median (R Core Team 2018).

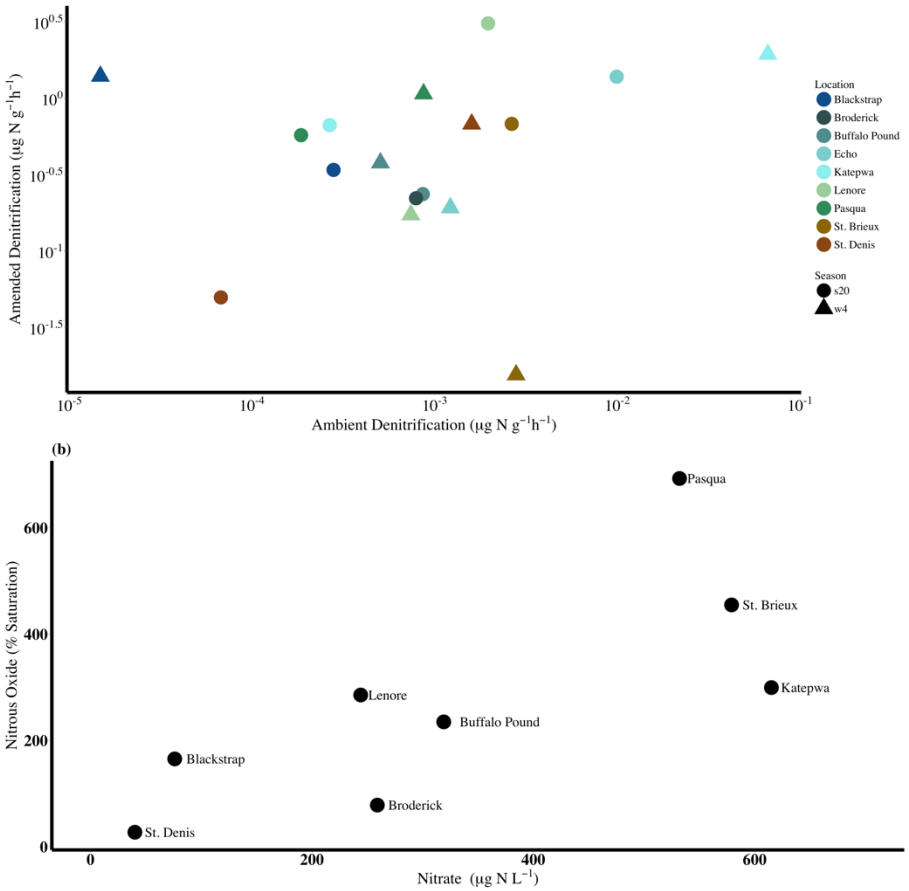

Figure 2-2 (a) Median denitrification rates at amended and ambient NO_3^- concentrations for winter and summer by location and (b) mean percent saturation of N_2O vs NO_3^- concentrations in winter in 9 lakes, reservoirs and ponds. The coefficient of variation for N_2O saturation is 14%.

Experimental results, comparing denitrification rates measured in winter at *in situ* (4ºC) and elevated temperatures (20ºC) showed no effect of temperature manipulation on winter denitrification rates (paired t-test, P = 0.55, 4 DF, Fig. 2-1a) when NO_3^- was maintained at natural concentrations. Likewise, denitrification rates did not differ between summer and winter when rates were measured at seasonal temperatures and ambient NO_3^- concentrations (paired t-test, P = 0.67, 7 DF, Fig. 2-1b). However, sediments showed a response to NO_3^- addition, which appears to mediate a temperature response. Nitrate-amended samples during winter showed a median 5-fold increase in denitrification rates when measured at elevated temperatures as compared to *in situ* temperatures (amended median denitrification winter

rates at 4°C and 20°C, respectively, were 0.27 and 1.34 μg N g^{-1} h^{-1}, paired t-test, P = 0.061, 4 DF, Fig. 2-1c). Likewise, NO_3^--amended samples in summer had a median 8-fold higher rates of denitrification than NO_3^--amended samples in winter (amended median denitrification winter rates at 4°C and summer rates at 20°C were 0.17 and 1.39 μg N g^{-1} h^{-1}, respectively, paired t-test, P = 0.086, 8 DF, Fig. 2-1d) – which contrasts from our results for ambient NO_3^- incubations. In summary, with the alleviation of NO_3^- limitation, it appears temperature may have a greater influence on denitrification rates. This was reflected in results of experimental temperature manipulation, and when we compared winter and summer rates (Figs 2-1 c,d). Median denitrification rates with NO_3^- amendment for both summer and winter more closely resemble literature rates from other regions than measurements at ambient NO_3^- concentrations (Appendix Tables A.2 & A.3). These higher rates likely imply that these systems are typically nitrate limited, as is common in the prairies as low in situ nitrate concentrations are fairly typical.

There was a large degree of variation in oxygen, conductivity, pH and nutrients across our 9 study systems. Seasonal differences were also apparent for some parameters (Figs 2-2 & 2-3, Appendix Table A.1). Bottom water NH_4^+ concentrations varied from 86 to 2250 μg NH_4^+-N L^{-1} and no difference was found between the winter and summer seasons (Paired Wilcoxon-Mann-Whitney signed rank test, P = 0.35, 14 DF, Fig. 2-3a). Bottom water NO_3^- concentrations varied from 57 to 615 μg NO_3^--N L^{-1}. Nitrate concentrations in winter were higher than summer concentrations (Paired Wilcoxon-Mann-Whitney signed rank test, P = 0.022, 14 DF, Fig. 2-3b). Bottom water N_2O percent saturation was highly variable – ranging from undersaturated to supersaturated, showing potential evidence of greater supersaturation in winter, which merits further study (Paired Wilcoxon-Mann-Whitney signed rank test, P = 0.12, 3 DF, Fig. 2-3c).

Oxygen concentrations varied during the study (near the bottom sediments) from little or no measureable oxygen to nearly 10 mg L^{-1}, while the average was near 4 mg L^{-1} (Appendix Table A.1). Conductivity varied from 372 µS cm^{-1} to 5072 µS cm^{-1} (average ~2200 µS cm^{-1}; Appendix Table A.1). Oxygen and conductivity did not show significant differences between winter and summer (Wilcoxon-Mann-Whitney signed rank test, P > 0. 05). The pH was higher in the summer and lower in the winter (Wilcoxon-Mann-Whitney signed rank test, P = 0.005, 14 DF) (Appendix Table A.1). Winter water temperatures near the sediments ranged from 0.9 to 4.3 ºC, and summer temperatures ranged from 11.1 to 21.1 ºC (Appendix A Table A.1).

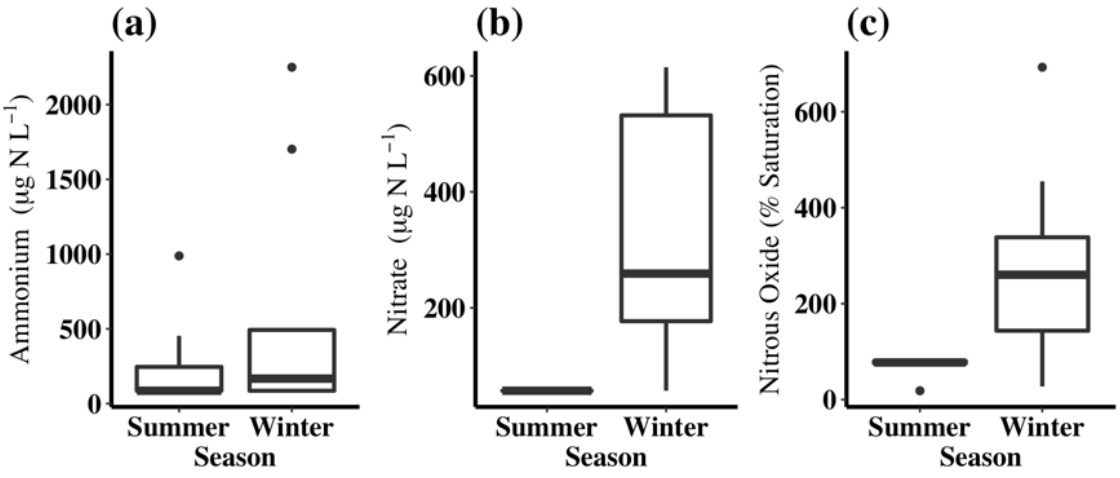

Figure 2-3 Ammonium, nitrate concentrations and N_2O percent saturation comparisons across 9 lakes, reservoirs and ponds between winter and summer seasons. The boxplot and whiskers encompasses 95% of the data observed, outside are outliers, inside the box are the first and third quartiles, and the center line is the median (R Core Team 2018).

2.5 Discussion

While we might assume that low temperatures will preclude active cycling – particularly for a biological process like denitrification that has an optimal range between 18 to 35ºC (Brin et al. 2017), here we demonstrate that winter denitrification rates rival summer rates despite low temperatures. This result is important for several reasons. First, it means that denitrification is active, and could affect nitrate availability in spring, and thus may be an

important control on spring diatom blooms (Bertilsson et al. 2013). Second, it lends insight into key differences in the nitrogen cycle between the ice covered and open water seasons. The ratio of winter to summer denitrification rates can reveal how the seasons compare (Appendix A Table A.2), even where dissimilar methods are used. Within this study, this ratio was greater than one (winter rates > summer rates) for four out of the eight study sites (Katepwa, Pasqua, St. Brieux, and St Denis), along with the nitrate-rich, ice-free Lake Suwa, Japan (Appendix A Tables A.1& A.2; Hasegawa and Okino 2004).

We observed higher concentrations of NO_3^- in winter months despite similar rates of denitrification across seasons. Sources of NO_3^- vary in winter (Fig. 2-4). Surface and groundwater inflows may also be important NO_3^- sources in some lakes during winter (Seitzinger et al. 2006; Costa et al. 2017), although these inputs are expected to be small in many of our systems which lack surface water inflows in winter, and where groundwater inputs are low (Pomeroy et al. 2005). Degradation of organic matter will continue to produce NH_4^+ through winter (Bertilsson et al. 2013) and nitrification remains active (Small et al. 2013; Powers et al. 2017a). While active denitrification should be a sink for some of this NO_3^- – low autotrophic uptake may be one of the most substantive winter changes in nitrogen cycling in many snow-covered lakes where light is limiting (Bertilsson et al. 2013). While the net effect appears to be the accumulation of NO_3^- (Powers et al. 2017b), here we demonstrate that this NO_3^- accumulation is not due to lower absolute rates of denitrification in winter. Interestingly, our denitrification rates were relatively low compared to literature values for the same method (Appendix A Table A.3). This is consistent with other measurements from lentic systems in the region (Gooding 2015) and may reflect relatively low NO_3^- concentrations, despite the large extent of agricultural land cover. Higher nitrate concentrations are typical of agricultural lands

heavily impacted by the application of fertilizers (Hansen et al. 2018), yet high nitrate was not apparent in this study.

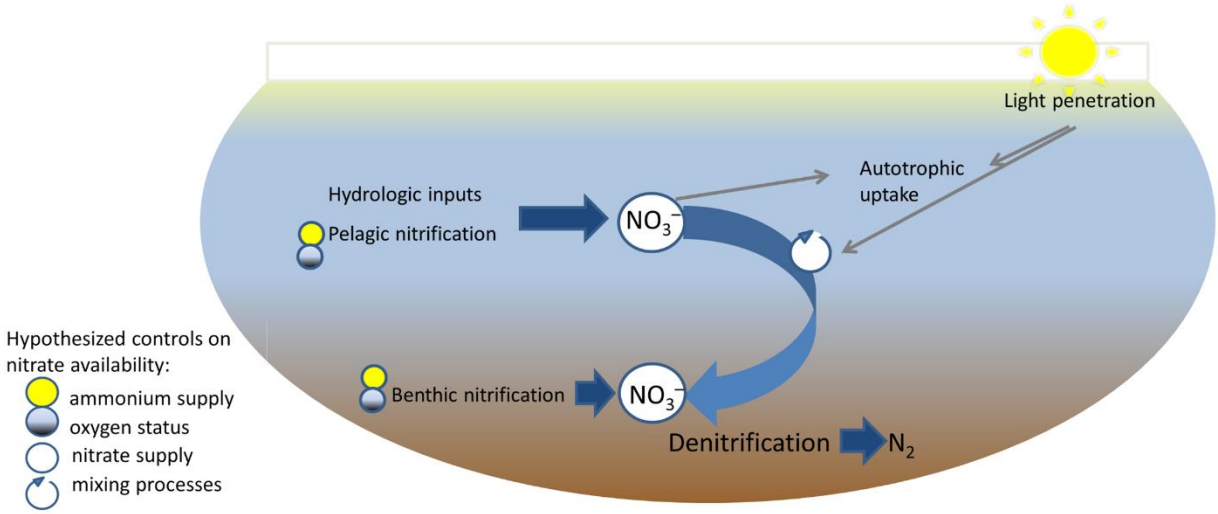

Figure 2-4 Conceptual diagram synthesizing key factors controlling winter **NO_3^-** that are likely to differ across lakes, with potential impacts on denitrification. These include hydrologic inputs (e.g., groundwater, melt events, autumn inflows), NH_4^+ supply (affecting nitrification rates), oxygen status in the water column (affecting pelagic nitrification) and at the sediment-water interface throughout the lake (affecting benthic nitrification), and the spatial separation of oxic waters from sediments (affecting the magnitude of mixing required to deliver pelagic NO_3^- to sediments) (Bertilsson et al. 2013; Costa et al. 2017; Hampton et al. 2017; Powers et al. 2017a; b). Mixing is likely to be an important control on NO_3^- supply to the sediments where the sediments are overlain by anoxic waters – which is common in many shallow-snow covered lakes through much of winter (Barica 1987). Nitrate uptake by autotrophs may also be important in lakes with high light penetration in winter. Given the importance of light in driving convective mixing (Pernica et al. 2017), the light environment may be an important control on oxygen, nitrification, NO_3^- uptake by autotrophs, and NO_3^- supply to sediments via convective mixing.

Our experimental results suggest that denitrification rates under ice in water bodies appear to be subject to hierarchical control. Nitrate concentrations represent a primary control and temperature a secondary control. One illustration of this impact is the Q10, the factor by which rates change over a 10°C change in temperature (Brown and Sibly 2012). Winter Q10 values are more variable than summer, but the overarching importance of NO_3^- supply is clear, with much higher Q10 for winter nitrate-amended denitrification rates (Appendix A Table A.2). Similarly, of our four sites where winter denitrification rates > summer rates, three sites (Katepwa, Pasqua, St. Brieux) had higher than average winter NO_3^- concentrations. Recent work in temperate marine water bodies also suggests the importance first of NO_3^- than temperature on governing

denitrification rates (Hasegawa and Okino 2004; Brin et al. 2017). Similarly, soil denitrification rates have been shown to respond positively at low temperatures when substrates are not limited (Breitenbeck and Bremner 1987). However, lower temperatures are associated with lower soil denitrification rates, and a higher yield of N gases (including N_2O) as intermediates or, side products (Knowles 1982).

Clearly there is more work to be done to understand changes in nitrogen cycling, and denitrification through winter – including work across all seasons, and with finer temperature manipulations, and reflecting smaller changes in NO_3^- as well as changes in organic carbon, using methods (like MIMS; An et al. 2001) that are more easily scaled to support model-based integration of our understanding of changing seasonal conditions. Ultimately, to understand winter changes in nitrogen, a more complete understanding of sources, sinks and cycling is required (Fig. 2-4). Changes in benthic nitrification rates, and coupled nitrification-denitrification across seasons could be particularly dynamic due to the potential for spatial separation of nitrification and denitrification in winter. Given the acetylene block method inhibits nitrification (Groffman et al. 2006), winter changes in coupled nitrification-denitrification cannot be assessed here. Because low oxygen conditions were common in the benthos of these lakes (6 of 9 water bodies in winter; 3 out of 9 in summer), we might anticipate that pelagic nitrification or littoral nitrification may have greater importance in many lakes, with convective mixing playing an important role in delivering NO_3^- to the sediments of shallow lakes (Fig. 2-4).

Our work shows a relationship between N_2O and NO_3^- under-ice, consistent with past work in rivers in winter (Baulch et al. 2011). This is also consistent with the hypothesis that nitrogen availability is a key determinant of N_2O production (Firestone and Davidson 1989). Yet, our results do not show a relationship between N_2O and denitrification rates, which is not necessarily

surprising given that the N_2O concentration is indicative of the whole winter season rather than current production/consumption (Soued et al. 2015). This lack of a direct relationship has been previously reported in small rivers (Harrison and Matson 2003), and may alternatively suggest that nitrification production of N_2O is also important. However, given the complexity of factors governing N_2O production and consumption (Firestone and Davidson 1989; Baulch et al. 2011), more work is clearly required to understand key processes, and controls. Our results in prairie ecosystems (Fig. 2-3c) combined with other recent work from boreal lakes (Soued et al. 2015) show that N_2O is typically supersaturated under ice. This raises important questions about the magnitude of a potential N_2O pulse at ice out. On the whole, despite the importance of aquatic emissions to global greenhouse gas budgets, lake emissions of N_2O are poorly constrained if considered at all (Ciais et al. 2013). Across our nine study systems, the mean winter percent saturation of N_2O was 280% (~587.3 $\mu g\ L^{-1}$ in excess of equilibrium values) while that of dissolved CH_4 was 721% (unpublished surface, data; ~2700 $\mu g\ L^{-1}$ in excess of equilibrium). Although more attention has been paid to ice-out fluxes of CH_4, if we consider the higher global warming potential (GWP) for N_2O of 298 compared to 34 for CH_4 (100y timescale; Myhre et al. 2013), it appears the potential for a large diffusive N_2O flux may have a greater impact than that of CH_4 at ice out from these systems. Many key questions remain, in terms of the relative importance of ice-out fluxes of these gases and, specifically, the extent to which the ponds re-equilibrate in spring will vary depending on wind-induced reaeration, and changes in production/consumption of both gases. However, it appears an ice-out pulse of N_2O may be important.

Interestingly, two of our study sites had low N_2O concentrations of 27% and 78% saturation in winter. This suggests, contrary to previous work, that N_2O is being consumed at low

temperatures (Baulch et al. 2011). Undersaturation of N_2O in winter was observed in systems with near sediment anoxia, conditions under which N_2O consumption by denitrifiers might be expected to occur. Summer undersaturation was also common, and was associated with low dissolved inorganic nitrogen, similar to past observations in aquatic ecosystems (Harrison and Matson 2003; Baulch et al. 2011; Soued et al. 2015). These data highlight fundamental questions regarding the conditions under which aquatic ecosystems can be net sinks of N_2O, and how frequently aquatic ecosystems were net sinks prior to anthropogenic disturbance. More broadly, given evidence that ice cover duration can affect N chemistry (Catalan 1992; Hampton et al. 2017; Powers et al. 2017b) and oxygen (Bertilsson et al. 2013), and nitrogen delivery is highly sensitive to hydrological change (Costa et al. 2017), there seems little question that the N_2O budget of lakes will be altered by climate change. However, the type of change may vary due to the multiple, spatially varying drivers, and controls. Given our summer data suggest a tendency towards N_2O under-saturation in these prairie water bodies, we hypothesize that ice-out emissions may offset a potential summer sink, but there is a clear need for detailed work across seasons, and in key periods such as ice-out to better constrain the role of freshwater bodies in global N_2O budgets.

2.6 Conclusion

Ice cover duration is declining in many areas, and is expected to continue to decline (Lemke et al. 2007). While we expected shorter periods of ice cover to impact nutrient cycling (Lemke et al. 2007), we found stability. This is demonstrated here by our finding that collective denitrification rates of the waterbodies are relatively stable between winter and summer months, despite major changes in water temperature in these temperate lakes. Experimental data suggest

that NO_3^- concentrations are the primary controls on denitrification rates. Given NO_3^- concentrations are higher in winter it appears higher NO_3^- may help to dampen any negative effect of lower winter water temperatures on denitrification rates. More work is needed to understand the role of coupled nitrification-denitrification in oxic sediments, and hydrological and hydrodynamic controls on NO_3^- delivery in systems with anoxic sediments. Our surprising finding of similar denitrification rates across winter and summer, also shown in an ice-free lake (Hasegawa and Okino 2004) suggests, given the potential for high export of NO_3^- during snowmelt in cold climates (Costa et al. 2017), that water bodies, where present in the landscape, may be able to attenuate NO_3^- loads even during the cold but critical snowmelt period. Our observation that N_2O is typically supersaturated under ice highlights the importance of ice-out monitoring to fully constrain aquatic greenhouse gas budgets. Clearly, many key questions remain about nitrogen and N_2O dynamics in the future and whether denitrification will become more, or less efficient in helping to mitigate current issues of excess nitrogen in the environment.

2.7 Acknowledgements

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2.8 Author contributions

HB and EC jointly conceived of the experimental design. EC performed the field work, laboratory experiments, statistical analysis (with advice from HB) and EC wrote and revised the manuscript with input from HB.

2.9 Transition statement

Chapter 2 and Chapter 3 span several temperate lakes, ponds and reservoirs. Chapter 2 demonstrates that winter denitrification rates did not differ from those in summer, despite large differences in temperature. Higher NO_3^- concentrations in winter appear to compensate for low-temperature suppression of rates, combining to minimize seasonal variation. Our work indicates that denitrification rates appear to be hierarchically controlled, first by NO_3^-, then by temperature. This suggests factors affecting NO_3^- delivery to sediments, including biological, hydrologic and hydrodynamic controls, may be more important than warming in controlling NO_3^- removal via denitrification. Chapter 3 will discuss NO_3^- production in winter via the process of nitrification which can be an important source of NO_3^- to denitrifying bacteria.

3.0 WINTER NITRIFICATION MATTERS, (SOMETIMES): MEASUREMENTS AND CONTROLS IN ICE-COVERED LAKES

Status: Submitted to PlosOne on October 5th, 2018

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3.1Abstract

With changes in ice cover duration, nutrient loading, and anoxia risk, it is important to understand the mechanisms that control both rates of nitrogen cycling and oxygen depletion in lakes through winter. Current understanding is largely limited to the description of changes in chemical species during winter, with few measurements of the processes driving winter changes, how they differ across lakes, and how they are impacted by under-ice conditions. Nitrification is a microbial process which consumes oxygen and ammonium (NH_4^+), and supplies nitrate (NO_3^-). To date, nitrification has been measured under winter ice cover in only one lake globally. Here, we used $^{15}NH_4^+$ enrichment to measure rates of pelagic nitrification in thirteen water bodies in two northern ecozones. Our work demonstrates that ecologically important rates of nitrification can occur despite low winter water temperatures, impacting NH_4^+, NO_3^- and, most importantly, oxygen concentrations. However, high rates are not the norm. When, where and why is nitrification important in winter? We found that nitrification rates were highest in a lake chain downstream of a wastewater treatment effluent, as well as in an oxic, semi-saline prairie lake.

In the boreal shield, a eutrophic lake had nitrification rates that exceeded those of an oligotrophic lake by 6-fold. Across all lakes, reservoirs and ponds, NH_4^+ concentrations were the strongest predictor of nitrification rates. While more work is required to understand the switch between high and low nitrification rates and strengthen our understanding of winter nitrogen cycling, this work demonstrates that high nitrification rates can occur in winter and are commonly associated with elevated NH_4^+ concentrations. As limnologists and managers continue to debate the need to control nitrogen within inland waters, winter merits further consideration, as nitrogen enrichment may be contributing to risk of seasonal anoxia, by stimulating nitrification rates.

3.2Introduction

Anthropogenic changes to the global nitrogen (N) cycle have led to significant increases in N inputs to rivers, lakes, oceans, and the atmosphere (Galloway et al. 2008). Within freshwater ecosystems, some of the most acute impacts of nitrogen fertilization are seen at sewage outfalls – where high NH_4^+ concentrations are nitrified (Leavitt et al. 2006; Ribot et al. 2012). The process of nitrification is a microbially-mediated one, whereby NH_4^+ is oxidized to nitrite (NO_2) then to NO_3^-, (Fig. 3-1; Ward et al. 1982). Nitrification leads to consumption of oxygen, which can be associated with fish kills (Magnuson et al. 1985; Powers et al. 2017a). In addition, because nitrification is the oxidation of NH_4^+, nitrification impacts the availability of different nitrogen species (Kemp and Dodds 2002), which can affect phytoplankton taxa and productivity (Glibert et al. 2016).

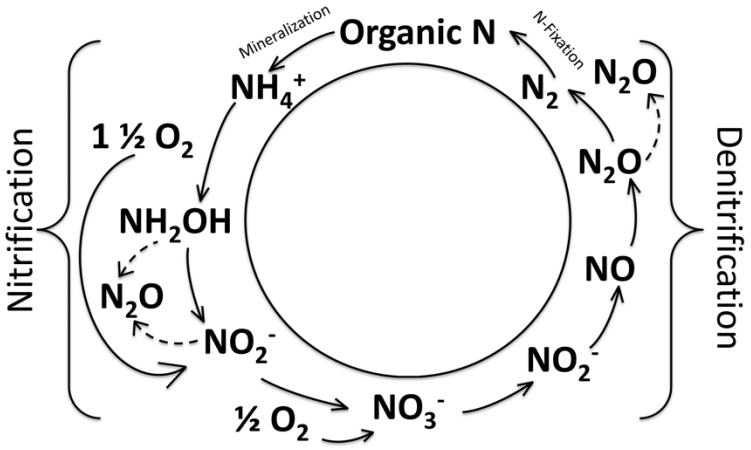

Figure 3-1 Schematic of the nitrogen cycle, featuring the microbial processes of nitrification (NH_4^+ to NO_2 and then to NO_3^-) and denitrification (NO_3^- to NO_2, nitric oxide, N_2O then to nitrogen gas). For every mole of NH_4^+ nitrified to NO_3^-, two moles of oxygen are consumed (Stoichiometric relationships collectively found in Allison 1973; Ward et al. 1982; Klingensmith and Alexander 1983; Wrage et al. 2001; Frame and Casciotti 2010; Powers et al. 2017a). Note that the proportion of N_2O released from nitrification and denitrification is highly variable as indicated by the dashed arrows (Firestone and Davidson 1989). Nitrogen assimilation, dissimilatory NO_3^- reduction to NH_4^+ (DNRA) and anaerobic NH_4^+ oxidation (anammox) are excluded from the figure but may be important components of the nitrogen cycle (Burgin and Hamilton 2007).

Nitrate generated from nitrification as a product, is a reactant for denitrification (Fig. 3-1). As such, nitrification can fuel the process of denitrification – a process which is considered an ecosystem service because it permanently removes fixed nitrogen. However, both nitrification and denitrification contribute to the production and emission of nitrous oxide (N_2O), a greenhouse gas and contributor to stratospheric ozone depletion (Fig. 3-1; Ravishankara et al. 2009). Recent work shows that N_2O supersaturation is common under ice (Soued et al. 2015; Cavaliere and Baulch 2018) and is indicative of active nitrogen cycling in winter. This conclusion of active N-cycling under ice is further supported by recent research demonstrating that winter denitrification rates are similar to those observed in warmer summer months (Cavaliere and Baulch 2018), and evidence from Wisconsin lakes that nitrification contributes to winter NO_3^- production and oxygen depletion (Powers et al. 2017a; b).

Nitrification may be the most important process in the nitrogen cycle to understand in winter due to its role in winter oxygen decline (Knowles and Lean 1987; Hosseini et al. 2017a; Powers

et al. 2017a). Substantive increases in winter NO_3^- in Wisconsin lakes are indicative of nitrification and reflect up to 25% of the oxygen decline (Powers et al. 2017a; b). Nitrification can be stimulated by increased substrate availability (Souza et al. 2014), suggesting that the potential for higher NH_4^+ availability (e.g., due to limited competition from autotrophs), could contribute to enhanced nitrification in winter (Powers et al. 2017b). Despite knowledge that the nitrogen cycle can be active under cold conditions, our understanding of biogeochemical cycling in the ice covered period is still in its infancy.

There are major physical, chemical and biological changes that might be expected to impact nitrogen cycling in ice-covered lakes (Hampton et al. 2017). Ice cover isolates lakes from the atmosphere, which leads to increased risk of hypoxia or anoxia in shallow, and snow-covered water bodies, driven both by aerobic respiration and nitrification (Barica and Mathias 1979; Knowles and Lean 1987; Powers et al. 2017a). Low light penetration can limit autotrophic activity and nutrient uptake (Bertilsson et al. 2013). Respiration (or mineralization) of organic matter continues, producing NH_4^+, which can either contribute to build up of this solute, or NH_4^+ may be consumed by nitrification (Catalan 1992; Hampton et al. 2017; Powers et al. 2017a). The low light conditions in winter may also be advantageous to nitrifiers (where adequate oxygen is available) because light can inhibit nitrification (Merbt et al. 2012; Bertilsson et al. 2013). However, the importance of this effect in winter is not known, as it is species specific, wave-length specific and dose dependent (Guerrero and Jones 1996).

Winter conditions may also slow down or inhibit nitrification. Low oxygen availability in winter (Mathias and Barica 1980) constrains nitrification rates in some ecosystems (Rysgaard et al. 1994). Typically, low temperatures are associated with low rates of microbial activity, and this is true for nitrifiers. Increasing temperatures have a positive impact on rates of nitrification,

particularly at moderate temperatures (10 to 35 °C; Stark 1996; Zeng et al. 2014). However, there is some evidence from work in the Arctic that nitrifying microbes can adapt to cold temperatures (Thamdrup and Fleischer 1998). Methane accumulation during winter (Canelhas et al. 2016; Denfeld et al. 2018) is also potentially important in controlling nitrification rates. Nitrifiers and methanotrophs have similar monooxygenases (Bédard et al. 1989) and as a result, methanotrophs can oxidize NH_4^+, much like nitrifiers can oxidize methane. Methane availability may affect nitrification rates via competitive inhibition (Bédard et al. 1989; Carini et al. 2003).

Quantifying the multitude of factors affecting nitrification is important to understanding current hypoxia risk, nitrogen cycling and future changes. Yet, few process-based measurements of nitrogen cycling in winter have been reported. Currently our understanding of nitrification in winter is limited to direct measurements in Lake St. George, Ontario, Table 3-1 (Knowles and Lean 1987), an isotope-based study of nitrification in Smith Lake, Alaska (Gu 2012), measurements in the cold, but ice-free Lake Superior (Table 3-2; Small et al. 2013) and estimates of nitrification and NO_3^- accumulation under ice from lakes in Wisconsin (Powers et al. 2017a; b). All of these studies suggest that nitrification can be important to oxygen decline in winter, yet this is a small number of measurements compared to the millions of seasonally ice-covered lakes globally (Verpoorter et al. 2014). Here we ask the questions: 1) What are pelagic nitrification rates under ice? 2) What factors are associated with high rates of winter nitrification? 3) Can winter nitrification be a significant mechanism for oxygen depletion under ice? and 4) Are nitrification rates correlated with N_2O accumulation under ice? We use measured rates from thirteen lakes, ponds, and reservoirs in Saskatchewan and northern Ontario, Canada (Fig. 3-2) which cross two northern ecozones to answer these questions supplementing our measurements with data from the literature.

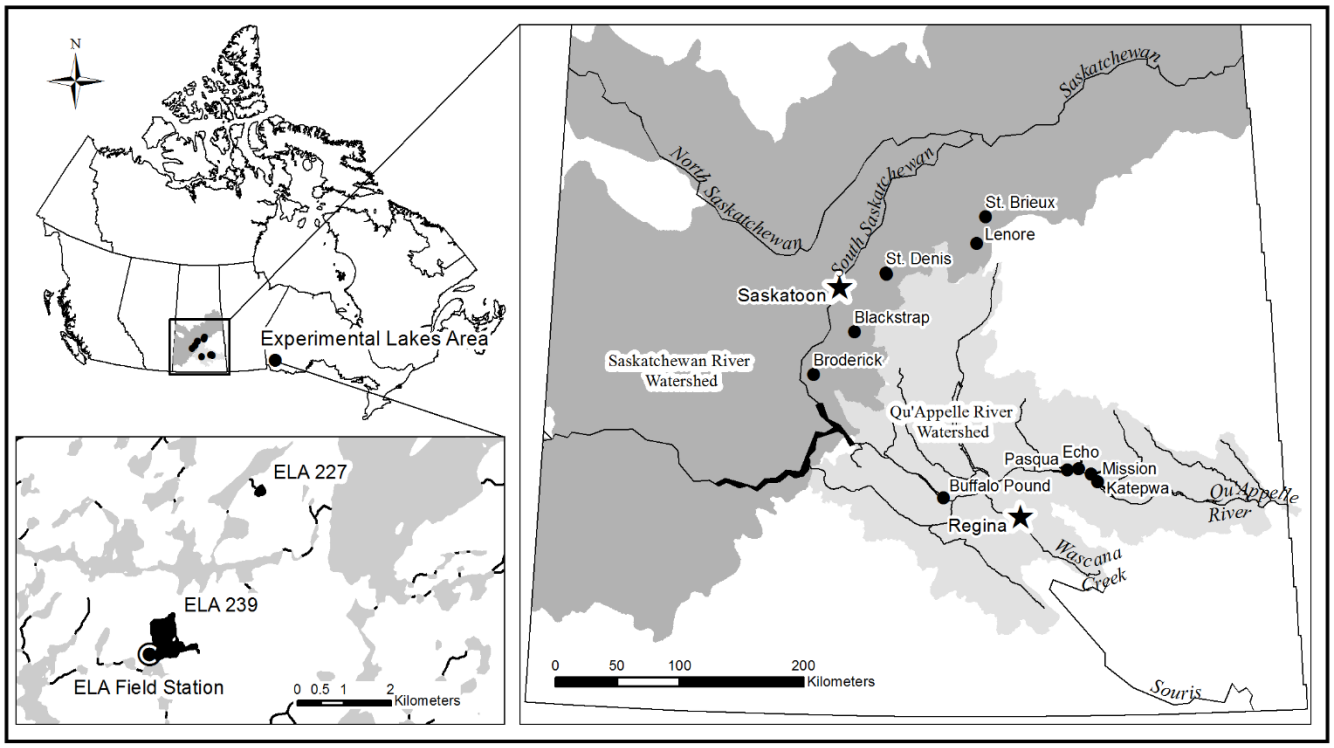

Figure 3-2 Map of Canada with overlays of Saskatchewan and Ontario study sites (map courtesy of Rosa Brannen). All Saskatchewan sites are in the prairie ecozone, while the Experimental Lakes area sites are in the boreal shield ecozone (Ecological Stratification Working Group 1995).

3.3 Methods

3.3.1 Study sites and sampling

Our study sites included 11 Saskatchewan lakes, ponds and reservoirs in the prairie ecozone (Fig. 3-2) and two lakes at the International Institute for Sustainable Development- Experimental Lakes Area (IISD-ELA) in northwestern Ontario, Canada (Boreal Shield; Fig. 3-2). The majority of samples were collected in March or February (Table 3-1). The St. Denis ponds were sampled in April during the melt period. The Saskatchewan water bodies include sites that are sources of drinking water, provide wildlife habitat and are important sites for recreation (Allan and Roy 1980; Pomeroy et al. 2005; Van Der Kamp et al. 2008). Buffalo Pound is a reservoir that is part of the Qu'Appelle system, upstream of Regina. Further along the Qu'Appelle chain are Pasqua,

Echo, Mission and Katepwa lakes. These four lakes are impacted by wastewater discharge from the upstream city of Regina as well as nearby agriculture (Leavitt et al. 2006). Three other water bodies are ponds in the St. Denis National Wildlife Area in a series of periodically connected ponds. Blackstrap is a reservoir, and St. Brieux and Lenore are interconnected lakes. These Saskatchewan water bodies are naturally mesotrophic or eutrophic (Allan and Roy 1980), and are impacted by human activities including agricultural land use and wastewater inputs. They face many challenges due to poor water quality, including oxygen depletion and degraded source water quality (Leavitt et al. 2006; Kehoe et al. 2015; Hosseini et al. 2017b). In contrast, Lake 239 at the ELA is a low nutrient (oligotrophic) lake while Lake 227 is naturally oligotrophic, but has been the subject of a multi-decade nutrient enrichment experiment altering the nutrient chemistry and trophic status over the past 40 years (Elser et al. 2002; Schindler et al. 2008). From 1969 to 1989 both N and P were added to Lake 227 then from 1990 to 2005 only P was added (Schindler et al. 2008).

Water samples and samples of dissolved gases were obtained in 2015 and 2016 by boring through the ice in each of the 8 Saskatchewan study sites. Samples for chemical analyses and nitrification experiments were obtained via peristaltic pump from a depth of 0.5 m below the ice-water interface into a plastic, acid-washed 20-L carboy in a heated tent (to prevent freezing in temperatures that frequently reached -30°C). *In situ* oxygen, temperature, pH, and specific conductance profiles at the time of sampling were collected using the YSI 556 Multi Probe System (YSI Environmental, Yellow Springs, OH) for all water bodies except St. Brieux and Lenore where the YSI ProPlus was used, courtesy of Dr. John-Mark Davies (Water Security Agency). Water and gas samples for the three St. Denis sites were collected via peristaltic pump, as well, but samples were obtained from shore due to unsafe ice conditions. Sampling of these

sites was achieved by anchoring hoses in the ice ~ 5-10m off shore (over ~2m of water) and using the pump and hoses to transfer water to the shore, where water, gas, and YSI measurements could be taken. This approach, necessitated by safety concerns, may contribute to elevated oxygen measurements at these sites. Dissolved gases (CH_4 and N_2O) were sampled via peristaltic pump by using headspace equilibrations after overfilling with sample water a 1.2-L glass bottle (Cole et al. 1994) in the Saskatchewan Lakes. Lakes 227 and 239 at the Experimental Lakes Area were sampled in a similar fashion for water, but were not sampled for CH_4 or N_2O. Experimental Lakes Area samples were obtained in March of 2016 by IISD-ELA (Ken Sandilands, biologist).

3.3.2 Laboratory and experimental methods

Water samples were protected from freezing and filtered upon return to the laboratory through pre-rinsed 0.2-µm polycarbonate filters (A.M.D. Manufacturing, Mississauga, Ontario) under low vacuum pressure. Subsamples of filtered water samples were analyzed for NO_3^- and nitrite (NO_3^- and NO_2 EPA method 353.2, hereafter referred to as NO_3^-), NH_4^+ (EPA 350.1), soluble reactive phosphorus (EPA 365.1), sulfate (Standard Method 426C) and alkalinity (EPA method 310.2) using the SmartChem 170 autoanalyzer (Westco Scientific Instruments, Inc., Brookfield, CT). Water samples were analyzed for NH_4^+ and soluble reactive phosphorus within 24 hours or less while water samples for sulfate and NO_3^- analyses were frozen after filtering, thawed and then analyzed. Alkalinity was measured on refrigerated filtered water. The water samples from ELA were analyzed upon receiving the shipped samples (within 3 days of sampling).

Headspace gas samples were analyzed for N_2O and methane, in duplicate, using the Scion 456 Gas Chromatograph (Bruker Ltd.). A micro-electron capture detector (ECD) was used to

measure N_2O and the flame ionization detector (FID) was used to measure methane. Dissolved N_2O and methane concentrations were calculated using standard solubility equations (Weiss and Price 1980). For semi-saline systems (St. Denis, St. Brieux and Lenore lakes), we calculated ionic salinity (as per Pawlowicz 2008) due to the importance of sulfate, calcium, magnesium and sodium ions. Filtered water samples were analyzed for ions using Inductively Coupled Plasma – Optical Emission Spectrometry, Department of Geology, University of Saskatchewan. These corrected salinity values were then used to determine dissolved concentrations and solubility of N_2O (Weiss and Price 1980) and methane (Wilhelm et al. 1977).

3.3.3 Nitrification experiment

Nitrification experiments were carried out as follows and as outlined in Carini and Joye (2008) adapted from (Ward 1987). In brief, water samples were analyzed for NH_4^+ on the SmartChem 170 Autoanalyzer, as noted previously (EPA 350.1). Unfiltered water samples were placed in Wheaton B.O.D. bottles, and samples were fortified with a spike of ^{15}N-enriched NH_4Cl (Sigma-Aldrich; 98 atom % ^{15}N, Lot # MBBB2704V) at a concentration equivalent to 10% of the measured NH_4^+ concentration. Samples were incubated at 4°C for 24 or 60 hours in the dark on a VWR DS-500 Orbital Shaker (Henry Troemner LLC, New Jersey). The 60-hour incubation time was to account for decreased activity at lower temperatures and to help maximize sensitivity of the method. We compared measurements from these two incubation periods (Wilcoxon-Mann-Whitney statistical test) to test for effects of incubation time on rate measurements, and found no significant difference between the two periods of incubation (wilcox.test, p-value = 0.125, 6 DF). Henceforth, only 60-hour incubation rates are reported. After incubation, the enriched water samples were filtered under low vacuum pressure through

0.2-μm polycarbonate filters to halt bacterial activity. The ^{15}N enriched water samples were used to calculate nitrification rates after $^{15}NO_3^-$ was recovered.

3.3.4 Nitrate recovery

Nitrification generates $^{15}NO_3^-$ in the experiments. In order to recover $^{15}NO_3^-$ from the sample water, the water samples were processed as follows using the NH_4^+ diffusion disk method outlined in Sigman et al. (1997), with minor alterations. The post-incubation filtrate was poured into sealable media bottles and magnesium oxide was added (MgO; pre-combusted at 650°C for 4 h). Samples were incubated at 65°C for five days to decompose dissolved organic nitrogen (DON) to NH_4^+. The media bottles were vented daily to release nitrogen gas. After the 5-day incubation, the water samples were boiled in the media bottles to remove NH_4^+ and reduce volume to below 100 mL. A NO_3^- spike of unlabeled NO_3^- was added if previously measured *in situ* NO_3^- concentrations were too low for analysis. The water samples were then adjusted to volume (100 mL) with deionized, distilled water. The water samples were then transferred back to the media bottles and sodium chloride was added (5 g) under recommendation by Dr. Sigman (pers. comm. D. M. Sigman; March 5, 2015). This protocol was developed for seawater (Sigman et al. 1997), and sodium chloride was added to avoid osmotic pressure on the diffusion packets and subsequent disintegration.

To recover the enriched NO_3^- (from nitrification) onto the diffusion disk, it must first be converted to NH_4^+. The diffusion disk (Teflon NH_4^+ diffusion disk packet, constructed using glass fiber filter paper sealed in Teflon tape with $KHSO_4$) was added to the media bottles with Devarda's alloy (100 mg Devarda's Metal Alloy; Fisher Scientific; Lot 137926) and bottles were sealed. This alloy reduces NO_3^- to NH_4^+, captured in the diffusion packet where the NH_4^+ ions

are converted to ammonia ions by the $KHSO_4$ and where it is kept in that form until analysis. The samples were incubated at 65°C for four days. Samples were then shaken on a VWR DS-500 Orbital Shaker (Henry Troemner LLC, New Jersey) for 7 days at 60 rpm. The Teflon packets were removed, rinsed in 10% HCl and then rinsed in deionized, distilled water and stored in scintillation vials until they were prepared for shipment by placing the diffusion disk in tin capsules. Samples were analyzed for ^{15}N using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) which was interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/).

3.3.5 Data and statistical analysis

Nitrification rates were calculated as per Sigman et al. (1997). After corrections were made for natural abundance and unlabeled N (as NO_3^- spike) and N addition due to Devarda's Alloy (as per Sigman et al. 1997), 46% of rates were below measurement thresholds. We calculated the limits of quantitation (LOQ) as follows: Using standard deviations of the ^{15}N (atom %) of the enriched samples (specific for each analysis), a method detection limit (MDL) was calculated by multiplying the standard deviation by Student t Distribution quantile (specific degrees of freedom). Then for each specific nitrification rate calculation we calculated the associated ^{15}N in micrograms (MDL in ^{15}N (atom %) × total N mass) for each sample and used that mass to calculate a minimum detectable rate based off the volume and incubation time for that sample. These LOQ rates range from 4.6×10^{-5} to 0.11 μg N L^{-1} d^{-1}. We assume that the low rates (< sample specific LOQ, in Table 2, reported with *) are due to low, un-measurable nitrification

rates rather than because of nitrogen recycling and possible underestimation of rates due to this recycling.

Due to the non-normal nature of the data, non-parametric tests were performed in R version 3.4.1 (R Core Team 2018). In order to assess links among different measured variables, a principal component analysis (PCA) was used (R Core Team 2018). The PCA shows how strongly related variables are by the proximity of the vectors – the more closely two (or more) variables are related – the closer those vectors will be in matching vector length and angle. To assess the measured variables (pH, oxygen, NO_3^-, and NH_4^+ concentrations, percent saturation of N_2O and methane) that could predict nitrification rates, linear model permutations (lmp) were used (Wheeler and Torchiano 2016a). To determine the best fit model, a general linear model was used to determine which set of variables make up the best model for predicting nitrification rates based on lowest (or best fit) AIC (Akaike's Information Criterion – AIC) for each model permutation. Next we supplemented our data with all available winter nitrification rate data from Lake Superior (a non-ice covered lake; Small et al. 2013), and Lake St. George (ice-covered; Knowles and Lean 1987) to assess relationships between NH_4^+ concentrations and nitrification rates. Finally, we divided the data into low ($< 1.1 \times 10^{-1}$ μg N L^{-1} d^{-1}) and higher ($> 1.1 \times 10^{-1}$ μg N L^{-1} d^{-1}) nitrification rates and used Signed Rank Mann-Whitney tests to understand the differences between these groups of data (wilcox.test; R Core Team 2018). This threshold value was selected because it represents the highest sample-specific LOQ (Table 3-1), and provides a reasonable separation between rates deemed to have little or no impact on nitrogen chemistry and oxygen consumption, and rates with a potentially important influence.

Table 3-1. Nitrification rates and associated concentrations of other variables for this study (under ice cover) for Lake St. George (near surface at 2 m depth, under ice cover; Knowles and Lean 1987); and for Lake Superior (near surface at 2 m depth, in winter but without ice-cover; Small et al. 2013). Values below LOQ for nitrification rates are reported, including negative values (following Small et al. 2013) and sample-specific LOQ (calculated as per Montoya et al. 1996; Peng et al. 2016) are reported.

Location	Nitrification Rate	Nitrification Rate LOQ	Ammonium	Nitrate	Oxygen	pH	Specific Conductance	N_2O	CH_4	Date
	($\mu g\ N\ L^{-1}d^{-1}$)	($\mu g\ N\ L^{-1}d^{-1}$)	($\mu g\ N\ L^{-1}$)	($\mu g\ N\ L^{-1}$)	($mg\ L^{-1}$)		($\mu S\ cm^{-1}$)	(% Saturation)	(% Saturation)	
Winter Ice Cover										
Blackstrap Reservoir	-1.7*	4.9×10^{-3}	<86**	<57**	7.6	5.7	1237	134.8	97.6	05-Mar-15
Buffalo Pound Lake	-4.0*	4.0×10^{-3}	<86**	<57**	12.5	7.6	1185	184.8	246.1	10-Mar-15
Echo Lake	1.2	7.0×10^{-2}	366	171	8.3	8.6	1726	213.7	241.5	23-Feb-16
Katepwa Lake	32.0	2.7×10^{-2}	<86**	620	9.9	6.8	1566	636.2	414.9	10-Mar-15
Lenore Lake	-1.7*	6.8×10^{-3}	263	200	7.5	8.4	3758	253	259.3	25-Mar-15
Mission Lake	2.1	1.1×10^{-1}	452	129	7.6	8.5	1790	195.4	282.1	23-Feb-16
Pasqua Lake	870.7	3.2×10^{-3}	1650	532	8.6	7.3	2180	671	178	10-Mar-15
St. Denis Pond 1	-0.042*	3.2×10^{-3}	<86**	<57**	10	6.9	1536	132.9	1109.3	16-Apr-15
St. Denis Pond 5338	-0.044*	3.1×10^{-3}	<86**	<57**	15	8.2	2963	135.7	1027.6	16-Apr-15
St. Denis Pond 90	-0.1*	3.6×10^{-3}	<86**	<57**	14.5	8.6	1648	109.1	1650.2	16-Apr-15
St. Brieux Lake	110.0	1.8×10^{-3}	528	310	2	7.8	3589	515.6	579.8	25-Mar-15
ELA Lake 227	0.14	4.6×10^{-5}	516	314	5.84	6.0	26	NA	NA	14-Mar-16
ELA Lake 239	0.022	1.2×10^{-4}	636	145	13.7	6.6	30	NA	NA	14-Mar-16
Lake St. George, Ontario	22.8	NA	467	540	9.3	NA	NA	NA	NA	27-Feb-80
Lake St. George, Ontario	12.2	NA	390	550	8.1	NA	NA	NA	NA	06-Mar-80
Lake St. George, Ontario	4.5	NA	65	1230	1.3	NA	NA	NA	NA	24-Feb-82
Lake St. George, Ontario	1.3	NA	39	1191	0.8	NA	NA	NA	NA	03-Mar-82
Lake St. George, Ontario	10.5	NA	490	210	10.2	NA	NA	NA	NA	09-Feb-83
Winter No Ice										
Western Basin of Lake Superior	0.34	NA	2.55	NA	NA	NA	NA	NA	NA	11-Nov-09
Western Basin of Lake Superior	0.03	NA	2.25	NA	NA	NA	NA	NA	NA	20-Mar-11
Minimum	-4.0	4.6×10^{-5}	2.25	57.0**	0.8	5.7	1185	109	98	
Median	0.8	3.6×10^{-3}	206	205	8.5	7.6	1648	195	282	
Maximum	870.70	1.1×10^{-1}	1650	1230.0	15	8.6	3758	671	1650	

*Indicates less than associated Limits of Quantitation for that sample. **Indicates that sample concentrations were below method detection limits of 86 and 57 $\mu g\ N\ L^{-1}$ for NH_4^+ and NO_3^-, respectively. NA indicates data were not reported.

3.4 Results

Our highest measured winter nitrification rates exceeded past winter measurements in lentic freshwaters (Fig. 3-3, Table 3-1), reaching rates as high as 870.7 μg N $L^{-1}d^{-1}$. Low, or unmeasurable rates were also very common – representing approximately half of our measurements (Fig. 3-4). Partitioning the nitrification rates into low (< 1.1 x 10^{-1} μg N L^{-1} d^{-1}) and higher (> 1.1 x 10^{-1} μg N L^{-1} d^{-1}) rates revealed that nitrogen species differed across these groups. Higher nitrification rates were associated with high NO_3^-, NH_4^+ and N_2O saturation (Fig. 3-4). Specifically, when nitrification rates were higher (greater than 1.1 x 10^{-1} μg N L^{-1} d^{-1}), median NH_4^+ concentrations were high at 483.5 μg N L^{-1}, while when rates were lower (less than 1.1 x 10^{-1} μg N L^{-1} d^{-1}) median NH_4^+ concentrations were also markedly lower (wilcox.test: P = 0.045, 11 DF, Fig. 3-4a). In addition, the higher nitrification rates (>1.1 x 10^{-1} μg N L^{-1} d^{-1}) were associated with higher median NO_3^- concentrations (312.1 μg N L^{-1}) while lower (< 1.1 x 10^{-1} μg N L^{-1} d^{-1}) nitrification rates were associated with lower median NO_3^- (57 μg N L^{-1}, wilcox.test: P = 0.005, 11 DF; Fig. 3-4b). Finally, when nitrification rates were greater than the LOQ of 1.1 x 10^{-1} μg N L^{-1} d^{-1}, median N_2O saturation (516% saturation) was nearly four-fold greater than when rates were lower than the LOQ of 1.1 x 10^{-1} μg N L^{-1} d^{-1} (median 135 % saturation; wilcox.test: P = 0.008, 9 DF; Fig. 3-4c). Nitrous oxide was supersaturated in the surface waters of all lakes, ponds and reservoirs, ranging from 109 and 671% saturation. This same threshold (nitrification rates less than or greater than 1.1 x 10^{-1} μg N L^{-1} d^{-1}) was not associated with differences in oxygen concentrations, methane percent saturation or pH (wilcox.test: P > 0.05). Across all data, the principal component analysis shows strong relationships among nitrification

rates, concentrations of NH_4^+ and NO_3^- and N_2O percent saturation (capturing 61% of the variability of the data; Fig. 3-5).

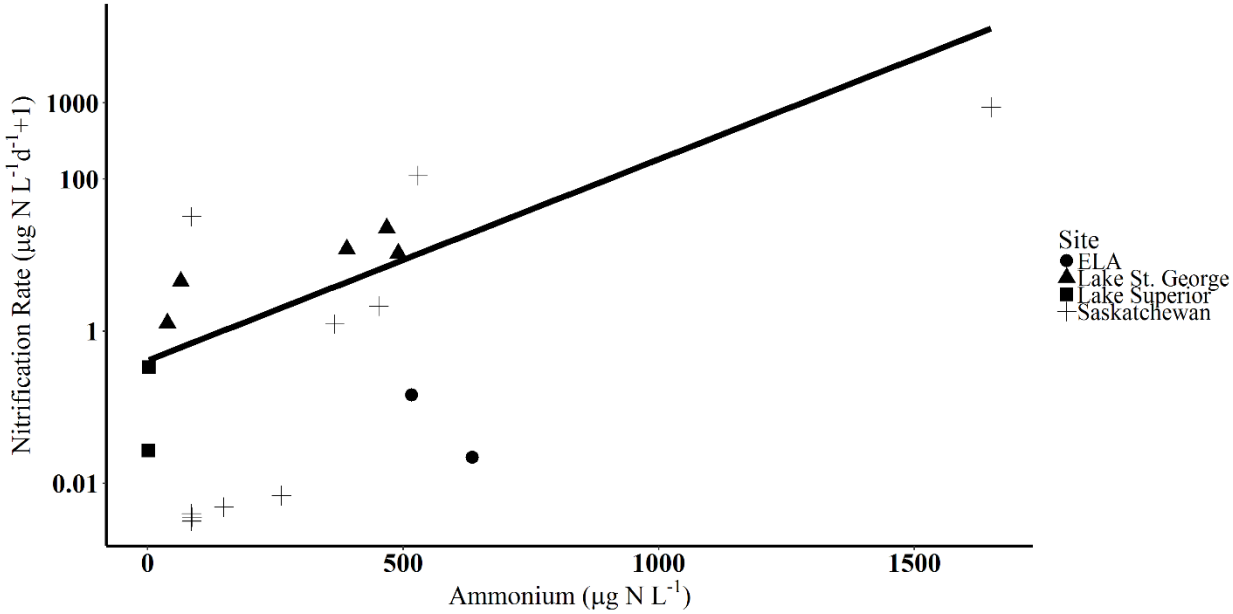

Figure 3-3 Relationship between nitrification rates and NH_4^+ concentrations for water bodies from this study (ELA and Saskatchewan) and other cold water measurements from Lake Superior (no ice-cover; Small et al. 2013) and Lake St. George (ice-covered; Knowles and Lean 1987). Note logged y-axis. The line plotted is the linear model (permutations) for all data from Table 3-1. The linear model and statistics are presented in Table 3-2. Nitrification rates less than their sample specific LOQ are replaced by their LOQ.

Table 3-2 Linear model relationships between nitrification rates (µg N L^{-1} d^{-1}) and NH_4^+ concentrations (µg N L^{-1}), using the linear permutations modeling approach.

Data usage	Linear model permutation	Model fit, significance, and degrees of freedom
All data (literature and this study)	Log(Nitrification rate) = $0.003 \times NH_4^+$-N – 0.39	Adjusted R^2 = 0.29, P < 0.001, 18 DF
All data (literature and this study, excluding Pasqua Lake)	Log(Nitrification rate) = 0.007 $\times NH_4^+$-N – 1.29	Adjusted R^2 = 0.09, P = 0.11, 17 DF
Only literature data	Log(Nitrification rate) =0.03 $\times NH_4^+$-N + 0.72	Adjusted R^2 = 0.57, P = 0.03, 5 DF

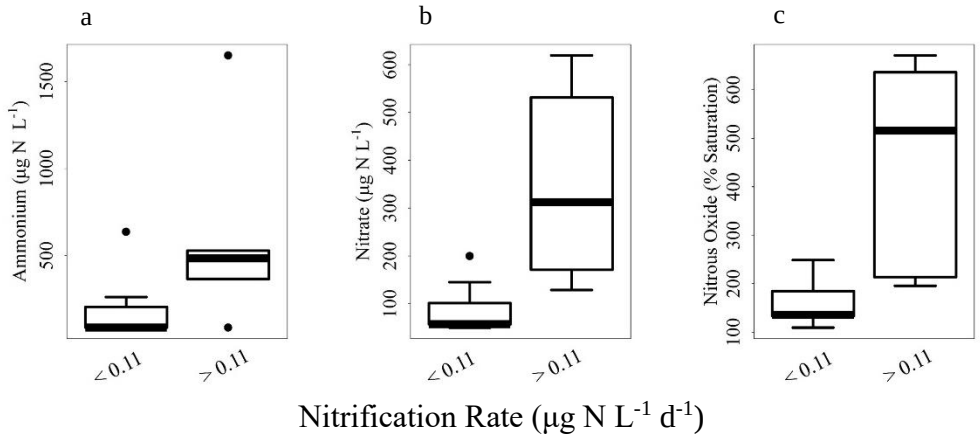

Figure 3-4 Concentrations of NH_4^+ and NO_3^-, and N_2O percent saturation partitioned according to nitrification rates that are above or below 0.11 µg N L^{-1} d^{-1}. There are significant differences between the two rate groups for all analyses (NH_4^+, NO_3^-, and N_2O; Wilcox-Mann-Whitney test, $P < 0.05$). The boxplot and whiskers encompass 95% of the data observed, data points outside of the box and whiskers are outliers. The box itself represents the first and third quartiles, and the center line is the median (R Core Team 2018).

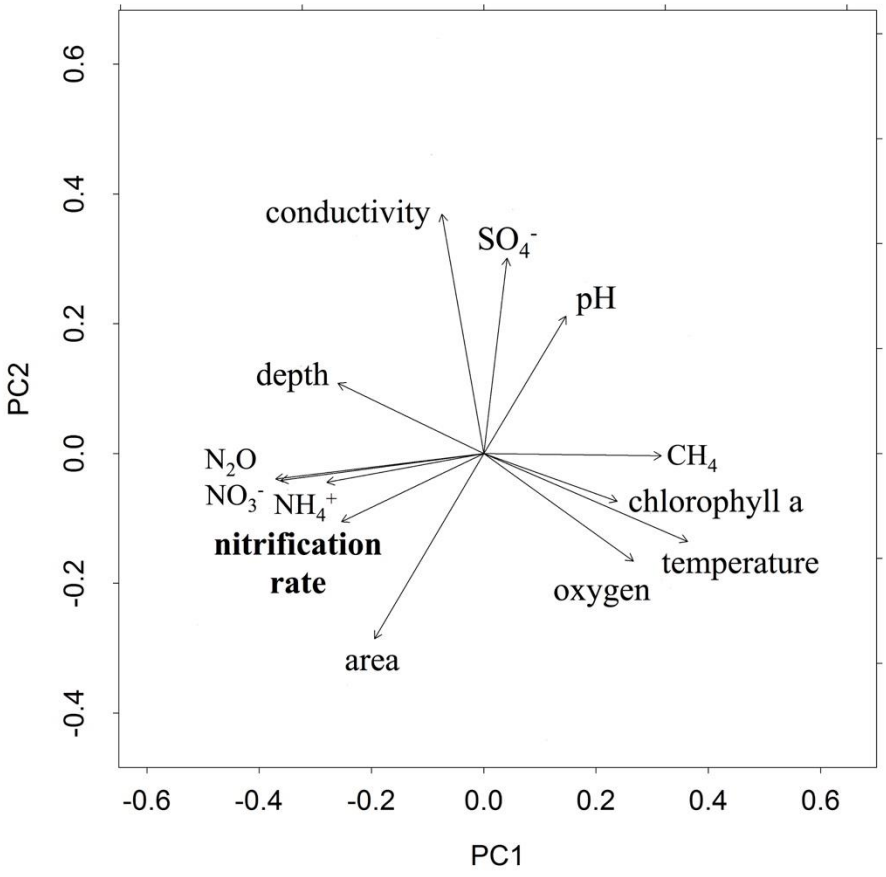

Figure 3-5 Principal component analysis showing the relationship between measured variables and nitrification rates. PC1 and PC2 account for 61% of the variance exhibited by the relationship among these variables. Within a PCA, the closer the component vectors are (angle and length) the more closely they are related. Note the association of nitrification rates with NO_3^-, N_2O and NH_4^+, and of methane with chlorophyll and temperature.

Literature data of nitrification rates were somewhat more restricted, hence our combined analysis is restricted to assessing relationships between winter nitrification rates and NH_4^+ concentrations. This analysis showed that across all lakes (this study, and Lakes St. George and Superior) where pelagic nitrification has been measured in winter, rates were significantly predicted by NH_4^+ concentrations (lmp, $P < 0.001$; adjusted $R^2 = 0.29$; 18 DF; Fig. 3-3 and Table 3-2). Our data show a strong influence of the high nitrification rate observed at Pasqua Lake (without Pasqua: lmp, $P = 0.09$; adjusted $R^2 = 0.11$; Table 3-2); however, we note that the literature data, alone, are indicative of a linear relationship (lmp; $P = 0.03$; adjusted $R^2 = 0.57$; 5 DF; Table 3-2).

Nitrate concentrations and N_2O % saturation were not linearly related to nitrification rates (lmp, $P > 0.05$), which, when combined with evidence of possible threshold effects (e.g., low nitrification rates associated with lower NO_3^- concentrations and N_2O % saturation), suggests non-linearity in the relationships, as might be expected where multiple factors (differential rates of nitrification and denitrification and yields of N_2O) influence these parameters. Interestingly, methane percent saturation, winter temperature and oxygen concentrations do not appear to be related to nitrification rates (Fig. 3-5, Table 3-2), although the range in variation in temperature was low, and oxygen concentrations were almost uniformly high.

Measurable nitrification rates within the prairies were predominantly found in Qu'Appelle lakes downstream of the outfall of the Regina Wastewater Treatment Plant. The outfall is released into Wascana Creek, then into the Qu'Appelle River, where the water enters, sequentially, Pasqua, Echo, Mission and Katepwa Lakes. While these wastewater-impacted lakes showed significant nitrification rates, they were not directly related to lake position or distance from the wastewater treatment plant outfall. The saline St. Brieux Lake also showed measurable

nitrification but the equally saline Lenore, which is presently connected to St. Brieux, did not. Within the boreal shield, nitrification rates were 6-fold greater in the experimentally eutrophied Lake 227 than the naturally oligotrophic Lake 239 despite both lakes having similarly high ammonia concentrations.

3.5 Discussion

Despite low temperatures, nitrification rates can be substantial in winter (Fig. 3-3, Table 3-1; Knowles and Lean 1987). Nitrification rates are also highly variable, suggesting some lakes will experience extremely high rates of nitrification-related oxygen consumption, while nitrification will have little or no impact in others (Figs. 3-1 & 3-3; Knowles and Lean 1987; Powers et al. 2017a). In systems like the treated wastewater-influenced Pasqua Lake, NH_4^+ concentrations exceeded 1500 μg L^{-1}, yet oxygen was available, and nitrification rates were exceptionally high (820 μg N L^{-1} d^{-1}). These nitrification rates are greater than previously reported in winter, and provide further evidence suggesting that nitrification is important under ice (Powers et al. 2017a), and that low water temperatures do not preclude active nitrification in lakes (Knowles and Lean 1987; Small et al. 2013) or other environments (e.g., biofilm reactor ponds; Young et al. 2017).

Our results, combined with results from the literature suggest that typically elevated NH_4^+ concentrations are associated with higher nitrification rates in winter, while some water bodies did not respond in predictable ways, i.e., more ammonium did not equate to higher nitrification rates (Lake 239, Lenore and Katepwa Lakes; Table 3-2, Fig. 3-3). High rates of respiration or nitrification can put water bodies at risk for anoxia, which is common in Saskatchewan water bodies due to often high organic carbon and nutrient concentrations, and lack of gas exchange through the long period of winter ice cover (Barica 1977). We can estimate oxygen depletion due

to nitrification using a mass ratio of 4.57:1 for O_2 consumed per NO_3^- produced (as per Powers et al. 2017a). The resulting median oxygen consumption rates across lakes are 110 μg O_2 L^{-1} per month –or 548 μg O_2 L^{-1} consumed over a 5-month period, which is substantial, but unlikely to markedly impact anoxia risk. However, the rate of oxygen consumption for the maximum nitrification rate (Pasqua Lake) was 1000-fold higher. This extremely high value represents a rate that can only be sustained based on substantial photosynthetic oxygen inputs, inflows, and/or gas exchange in areas of open water. Likely, this rate represents a hot spot, or hot moment of nitrification that is not representative of the whole winter period or whole lake, which is not known to undergo winter anoxia or fish kills. Oxygen concentrations were high at the time of sampling, associated with very little snow. While inflows into Pasqua Lake were low at the time of sampling (Environment and Climate Change Canada; Station Number 05JK007), this inflow may also have brought oxygen to the lake helping balance high oxygen demand. Finally, experimental conditions, specifically, dark incubations, may have favored enhanced rates of nitrification, by removing any potential light inhibition (Guerrero and Jones 1996; Merbt et al. 2012; Peng et al. 2016). Nonetheless, this high rate has the potential to have a major short-term effect on oxygen concentrations.

Our results, combined with results from the literature suggest that elevated NH_4^+ concentrations are associated with higher nitrification rates in winter. This highlights the importance of anoxia risk assessment and management efforts to limit the export of NH_4^+ and organic matter in wastewater effluents, even during winter – a period, which can pose significant technical challenges to wastewater treatment plants. This need to limit the release of oxygen-demanding substrates has been known for decades (Fair 1939; Arbabi et al. 1974). However, there are subtleties here that necessitate consideration in the current debate regarding the need for

nitrogen management of inland freshwaters (Schindler 2006; Lewis et al. 2011). Systems with higher nitrogen loads, even where effluents are nitrified and effluent oxygen demand is effectively controlled may still have high mineralization rates, leading to elevated NH_4^+ availability.

We demonstrate the potential for high nitrification rates, but this is not uniformly observed – with low, or unmeasurable rates being more common. Within ice-covered lakes, it is likely that hotspots of nitrification occur, where NH_4^+, oxygen and nitrifying bacteria meet. In some systems, where oxygen is present near the sediment-water interface, benthic nitrification may also be an important process (Rysgaard et al. 1994). The shallow water depths of many prairie water bodies means that the sediments have a greater impact on water chemistry, particularly to NH_4^+ and oxygen concentrations (Scheffer et al. 1993; Müller et al. 2012), although benthic anoxia may limit the role of sediments in nitrification. Another potentially dynamic region may be the near-surface zone, as demonstrated by previous winter work in Lake Superior, where both high and low rates were commonly observed through the year (Small et al. 2013). The depth maxima of nitrification rates in lakes may differ markedly through seasons (Small et al. 2013); suggesting detailed study of both spatial and temporal change in nitrification rates across lakes may be required to fully understand oxygen consumption and nitrogen cycling. In the context of winter, this may be particularly important, and challenging, as transient mixing events (Bertilsson et al. 2013; Pernica et al. 2017) may be a key trigger of increased nitrification rates.

Winter nitrification has the potential to impact the speciation of dissolved inorganic nitrogen at ice out, with possible impacts on phytoplankton communities. For example, diatom dominance is favored when there is more available NO_3^- and community dominance transitions to other species when NH_4^+: NO_3^- increases (Glibert et al. 1995, 2016; Lomas and Glibert 1999). Further,

winter availability of NO_3^- via nitrification also links nitrogen removal via denitrification, which remains active in winter (Fig. 3-1; Seitzinger et al. 2006; Cavaliere and Baulch 2018). More work is required on the nitrogen cycle in its entirety to better understand factors controlling dissolved inorganic nitrogen concentrations and winter changes which may affect the spring bloom across the millions of temperate lakes globally (Verpoorter et al. 2014).

Winter appears to be an important time for N_2O generation and build up under ice-cover (Soued et al. 2015). Nitrous oxide was supersaturated in all water bodies, which reflects active nitrogen cycling and the trapping of N_2O under ice-cover, although we have periodically observed undersaturation near the sediments (Cavaliere and Baulch 2018). The observation that higher rates of nitrification are linked to higher N_2O concentrations is not surprising, given N_2O is produced as a result of nitrification. Indeed, as much as 25% of NH_4^+ may be converted to N_2O by nitrifying bacteria (Jørgensen et al. 1984), although yields are often much lower. Because our measurements are more typical of mid-late winter conditions and not the ice-out period, more work is required to understand changes through winter affecting the speciation of dissolved nitrogen, and, processes affecting N_2O production, and consumption. Given many lakes in later winter may have high NH_4^+ concentrations (Barica 1977), this may further increase the risk of a significant ice-out N_2O emissions pulse (Soued et al. 2015).

Our work is also relevant to eutrophication management. While winter hypoxia risk is only one consideration in eutrophication management, this is an area where the effects of nitrogen management are not well understood, but may be particularly important. Interestingly, within the prairie ecozone, nitrification rates were measurable only in two types of lakes: a hypoxic, semi-saline lake (St Brieux), and lakes downstream of a wastewater treatment plant (sequentially: Pasqua, Echo, Mission and Katepwa Lakes), that are situated in a catchment with extensive

agricultural activity. Since nitrification rates from this study were measured in the Qu'Appelle chain of lakes in winters of 2015 and 2016, there has been a significant modification to the Wastewater Treatment Plant in Regina in the form of tertiary treatment of the waste water. This is expected to lead to reduced concentrations of total nitrogen entering these water bodies. While the oxygen concentrations at St. Brieux could limit nitrification in the field, there is the potential that introduction of oxygen during sampling may have contributed to these elevated rates. Alternatively, this may reflect nitrification under low oxygen conditions, which is known to occur, and is often associated with higher N_2O concentrations, as observed here (Table 3-1; Jørgensen et al. 1984). Within the boreal shield, nitrification rates of an experimentally eutrophied lake exceeded those of a naturally oligotrophic lake by 6-fold. More work is required to better understand the importance of nitrogen management to nitrification rates, and associated oxygen demand; however, these results may suggest a positive relationship between eutrophication and nitrification rates even in the absence of an immediate point source input.

3.6 Conclusion

Our work demonstrates that the high nitrification rates can contribute to winter oxygen decline, particularly when NH_4^+ concentrations are high. This suggests that the practice of permitting higher concentrations of NH_4^+ in wastewater effluent during winter months (Water Security Agency 2015), could contribute to increased winter nitrification oxygen demand. While these higher winter effluent limits reflect technical and cost constraints, this also reflects a period of high ecological sensitivity. Fortunately, the recent upgrade of the waste water treatment plant in Regina has led to decreased nitrogen and ammonia discharge and hence may help reduce the risks of winter oxygen depletion due to high rates of nitrification. Nitrification rates influence the

production of NO_3^-, and N_2O in winter; hence this process represents a key understudied control on lentic N_2O budgets, and a key control on the availability of different nitrogen species at ice-out. Our work demonstrates nitrification can be important in winter, but further work is needed to understand the drivers that lead to hot moments and hot spots of nitrification-associated oxygen depletion and N_2O production in ice-covered water bodies.

3.7 Acknowledgements

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3.8 Author contributions

E.C. and H.B. developed the study design. E.C. first developed the method as described then performed the field work, the laboratory analysis, data analysis and wrote the manuscript. H.B. advised at all points during the work and provided edits on the manuscript.

3.9 Transition statement

Chapter 2 and 3 provided insight into key nitrogen transformation processes. Chapter 4 focuses on understanding changes in pond chemistry within prairie potholes through winter, spring melt, and into the open water period. This work integrates process-based measurements of nitrification and denitrification and builds a more complete understanding of how chemistry changes, and the processes driving these changes as winter transitions to spring. Building on insights gained in chapters 2 and 3, greenhouse gases are measured in these ponds to better understand controls on spring gas efflux.

4.0 THE RISE AND FALL OF NUTRIENTS IN ICE COVERED PONDS

Status: In preparation

Citation: Emily Cavaliere, Helen M. Baulch, G. Koehler. In preparation. The rise and fall of nutrients in ice covered ponds

4.1Abstract

Small ponds are numerically abundant throughout the temperate zone, particularly within the Prairie Pothole region. Winters in this region can be long, and the winter period of ice cover is one of many key changes. Ice-cover isolates ponds from the atmosphere leading to the occurrence of low oxygen conditions in the long, unique, and under-studied period winter period. Winter is followed by a period of rapid physical change as spring-melt begins. Winter and spring-melt periods were defined by identifying, through breakpoint analysis, key periods of changes in conductivity: the period of increasing conductivity is identified as winter and spring-melt as the period after conductivity stops increasing and begins to decrease. Here we undertook an intensive field campaign, studying prairie pothole ponds through winter, spring-melt, and open water to understand the biogeochemical transformations that control pond nutrients and greenhouse gases in shallow ponds (mean pond depth: 2.5 m; mean surface area: 386,000 m^2). Through winter, we observed the accumulation of ammonium (NH_4^+) and soluble reactive phosphorus (SRP) and concurrent declines in nitrate (NO_3^-) concentrations.

The shift from winter to the onset of spring-melt prompts a dramatic physical change that alters oxygen, light, and solute concentrations. At the onset of melt conditions NH_4^+ and SRP concentrations decline, despite nutrient-rich melt water inputs. This shift in conditions is associated with the increased availability of light, and oxygen, which appear to cause a 'spring biogeochemical switch.' During the spring-switch, nutrient uptake rates increase associated with higher light, oxygen availability increases associated with photosynthesis, atmospheric gas exchange, and geochemical processes are likely to contribute to declines in SRP. Spring melt led to a 36% decrease in SRP, and 50% decrease in NH_4^+. Ammonium uptake by plankton increased 18-fold from winter to melt, and mean NO_3^- uptake during the spring-melt period exceeded that of the open-water period. Rates of denitrification did not appear to undergo this shift. Instead, denitrification rates were similar across all periods and showed strong control of NO_3^- availability. Interestingly, nitrification rates during spring-melt were low despite the availability of NH_4^+ and oxygen. Together, these observations, demonstrating rapid changes in both nutrient concentrations and the rates of some processes support the narrative that small ponds and wetlands act as a hotspot for nutrient transformations despite their small size. Importantly, the spring is a key hot moment. Ponds can assimilate the high inputs from the catchment, and show declines in inorganic nutrient concentrations. The hot moment co-occurs with the period of maximum potential for hydrologic connectivity, associated with the risk of 'fill and spill' during snowmelt. This suggests that the spring ice-out period, and associated injection of oxygen and light are protective of downstream ecosystems, and decrease the downstream transport of dissolved nutrients. This also suggests a refocusing is required from understanding implications of declining periods of ice cover, to understanding changes within these biogeochemically unique periods. We conclude that the spring biogeochemical shift may help buffer some of the impacts

to downstream ecosystems resulting from decreased periods of ice cover if dissolved inorganic nutrients are effectively retained or transformed within the wetland ponds. However, more work is required to understand the fate of nutrients accumulated within biomass during spring bloom.

4.2 Introduction

Small, shallow ponds and lakes are ubiquitous on the landscape. While they are often excluded from landscape-scale biogeochemical budgets, they can play a large role in nutrient cycling (Downing 2010; Cheng and Basu 2017). Within the temperate zone, they experience winter ice cover for a period that can exceed six months (Fig. 4-1). The shallow and highly productive nature of small ponds combined with isolation from the atmosphere (preventing gas exchange), and a cessation of surface flows, sets up conditions for oxygen depletion (Barica and Mathias 1979; Catalan 1992; Agbeti and Smol 1995; Hampton et al. 2017).

Low oxygen conditions are associated with many biogeochemical and ecological changes including changes in nitrogen cycling, phosphorus cycling, and greenhouse gas production (Guenther and Hubert 1991; Meding and Jackson 2003; Kirillin et al. 2012; Bertilsson et al. 2013). However, these winter changes are largely unstudied, leaving major gaps in our understanding of pond and shallow lake nutrient dynamics, and also in regional hydrochemistry (Fig. 4-1). Ponds and other lentic ecosystems receive nutrients from a variety of sources, including atmospheric deposition, runoff, local terrestrial inputs and groundwater (Carpenter et al. 1998). During ice cover, the external nutrient supply is limited due to a decline or cessation in inputs from most external sources (Bertilsson et al. 2013). Under ice-cover, nutrient dynamics reflect a balance between algal and microbial uptake, and mineralization (Catalan 1992; Meding and Jackson 2001; Bertilsson et al. 2013), with the

balance of these processes expected to vary over time due to the changing chemical and physical conditions.

Low light conditions in winter are dependent on the type of ice and the amount of snow and ice. If there is little to no snow and clear ice, light can penetrate to the water below (Bengtsson 1996), stimulating phytoplankton growth, which can be significant under ice-cover (Catalan 1992; Gu and Alexander 1993). Light penetration can heat the water below, causing mixing (Bertilsson et al. 2013; Pernica et al. 2017), and altering the light environment for phytoplankton. Winter oxygen sources are constrained to photosynthetic production while the drivers of consumption include the processes like organic matter decomposition, nitrification and methanotrophy (Knowles and Lean 1987; Catalan 1992; Denfeld et al. 2016a). The sediment-water interface, particularly in shallow water bodies, is likely the site of rapid oxygen depletion by sediment processes causing frequent anoxia or hypoxia (Meding and Jackson 2001, 2003; Bertilsson et al. 2013).

Oxygen is important for both nitrogen (N) and phosphorus (P) cycles, particularly in winter as oxygen replenishment is limited. Aerobic conditions favor nitrification and P sorption (Pauer and Auer 2000; Kleeberg et al. 2013) and lower concentrations of oxygen, particularly at the sediment-water interface, can enhance N removal via denitrification and P release from oxidized iron complexes (Knowles 1982; Nürnberg 1984). Winter work, while rare, tends to show DIN accumulation over winter (Hampton et al. 2017). Nitrate accumulation has been shown in some studies (Hampton et al. 2017; Powers et al. 2017b; Cavaliere and Baulch 2018), while reports of ammonium accumulation appear more limited (e.g., within an eutrophic lake; Gu and Alexander 1993). Due to low oxygen concentrations, we might expect higher redox-sensitive P release rates

from sediments in winter. While higher winter internal loading may be rare (Boström et al. 1988; D'Silva 2017; Orihel et al. 2017), evidence from a large reservoir suggests that winter P release is important (North et al. 2015), and preliminary work suggests there is potential enhancement of anoxic sediment phosphorus release rates within prairie potholes (Armstrong 2018). We lack a full understanding of where winter P release is important, and where it might remain low through the winter season (Boström et al. 1988; D'Silva 2017; Orihel et al. 2017).

Given the importance of benthic habitats to nutrient cycling on the whole, shallow ponds cycle nutrients more quickly than deeper lakes (Mathias and Barica 1980). These ponds might be the location of the most rapid changes in winter, particularly due to frequent and sometimes long-term winter low-oxygen conditions. Despite their ecological significance on the landscape (Mushet 2016), wetland numbers are on the decline, largely attributed to the practice of drainage (Waz and Creed 2017).

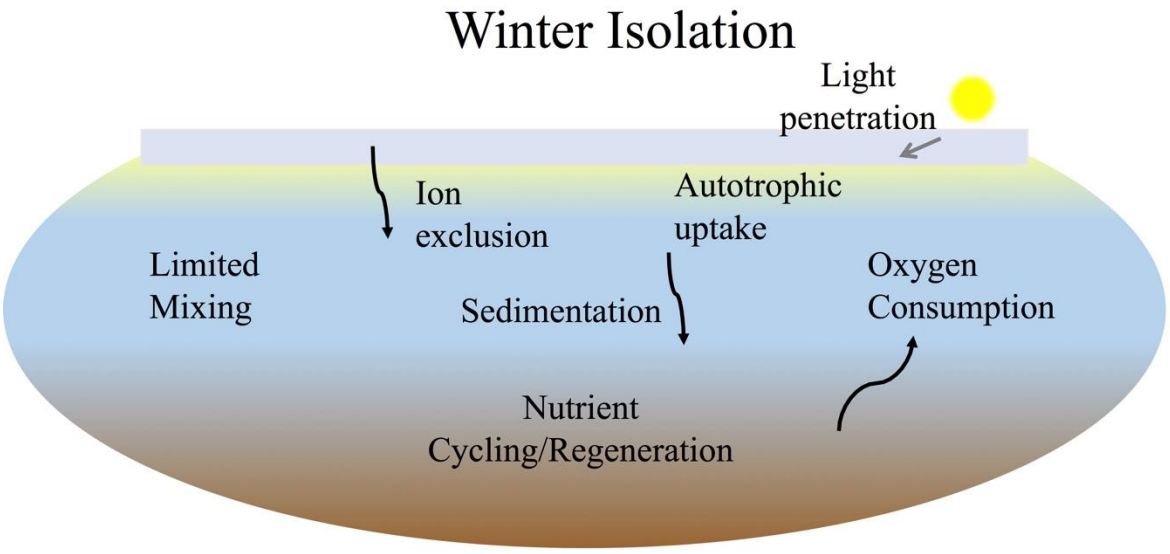
Prairie water bodies, like water bodies globally, experience nutrient pollution. Increased concentrations of nutrients can cause corresponding increase in growth of phytoplankton sometimes leading to a community shift towards the dominance of cyanobacteria and increased anoxia risk (Jackson et al. 2001; Meding and Jackson 2003; Schindler 2012). Wetlands, and drainage of wetlands may have complex impacts on nutrient loads. While wetlands can contribute to nutrient retention (Mitsch 1995; White and Bayley 2001), the export of solutes from nutrient rich wetlands can cause negative effects downstream (White and Bayley 2001) and will be affected by changing hydrologic regime (Fang and Pomeroy 2008) and potentially also impacted by changing ice-cover duration (Fang and Stefan 1996).

Winter is important because it precedes the key period of runoff generation during snowmelt. Prairie hydrology is such that during the spring-melt, the confluence of high nutrient concentrations and large quantities of water could move winter accumulated nutrients into wetland ponds (Cade-Menun et al. 2013; Costa et al. 2017). In the prairie pothole region, wetland ponds are subject to 'fill and spill' dynamics (Phillips et al. 2011; Shaw et al. 2012) – meaning that wetlands show transient connectivity, and can be transient contributors to streamflow. The timing of fill and spill is important because it often happens during spring-melt when snow and ice fill wetlands (Hayashi et al. 2016). This suggests that winter and spring biogeochemical conditions within ponds may control downstream solute export (Leibowitz and Vining 2003; Hayashi et al. 2016). As the weather warms in spring, an influx of nutrients from runoff during snowmelt is expected (Petrone et al. 2007; Cade-Menun et al. 2013). At the same time, oxygen is reinjected into the ponds via surface runoff and gaps in the ice cover (Catalan 1992). As a result, we expect nutrient cycling to be highly dynamic with the influx of nutrients from the catchment, a shift from anoxia to oxic conditions, and much higher light availability which will support an increase in aquatic productivity (Bertilsson et al. 2013). This means that major biogeochemical changes are expected to co-occur with key hydrologic periods. As such, snowmelt and ice-out are expected to be complex and interesting 'hot moments' of change within prairie potholes.

Clearly, understanding current conditions during the ice cover, spring-melt, and ice-off periods is important to understanding pothole ecosystems themselves, and their role in catchment nutrient export. Here, we explored changes in biogeochemistry of three prairie pothole wetland ponds (referred to as wetland ponds) across three winter periods with detailed sampling during the winter and spring-melt period. Our sampling program was designed to help understand the interplay between in-pond processes, and catchment-driven change, aided by measurements of

stable isotopes of water, nutrient concentration, and process-based measurements of N cycling. Specifically, we asked: Are winter and spring-melt periods an important time of biogeochemical change? How and why do nutrients change within these periods? And finally, what are the roles of different biogeochemical processes in driving changes in nitrogen during winter and the spring-melt period?

a.



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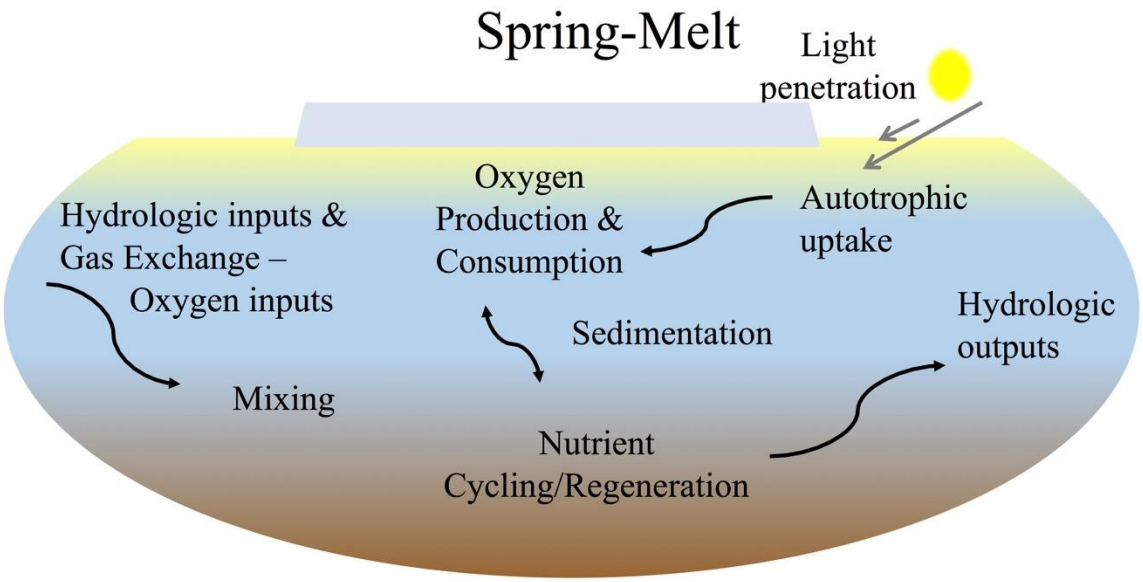

Figure 4-1 Wetland ponds experience unique conditions in winter and spring-melt. a. Winter is characterized by isolation from the atmosphere, low light and consequently low autotrophic uptake. However, mineralization continues despite the cold temperatures and nutrients continue to regenerate. Ions are excluded as the ice forms. b. During spring-melt, the isolation ends, light penetration increases as do the hydrologic and gas inputs, while sedimentation and nutrient regeneration likely remain the same. The spring-melt period begins as light and ice-cover alter with warming. These periods are difficult to study as they are cold and during melt, the ice cover becomes unsafe. Both the winter isolation and spring melt periods experience dramatic swings in oxygen, light availability, hydrologic and gas inputs and the potential for nutrient fluxes that require further study.

4.3 Methods

4.3.1 Study site

The prairie pothole region has many, small ponds that contribute to the biogeochemical processing of many of the agricultural and urban inputs of the region (Cheng and Basu 2017). Our work was situated at the St. Denis National Wildlife Area (SDNWA), a long-term study site located near Saskatoon, SK (Dzus and Clark 1998; Hayashi et al. 2003; van der Kamp et al. 2003; Ma et al. 2008; Fig. 4-2). While the area includes wetland classes that range from ephemeral to permanent (Hayashi et al. 2016), our work is centered in three permanent wetland ponds (ponds 1, 90 and 5340) which vary in mean depth from 1.4 to 4.1 m and have ice-cover annually from November until April or May. These wetlands are impacted by agricultural activity in the surrounding farmland and are high in nutrients (summer TP = 367 $\mu g\ L^{-1}$; winter TP = 340 $\mu g\ L^{-1}$), organic carbon (average DOC range: 20-103 $mg\ L^{-1}$;Waiser 2006) and have a range of salinities (observed range: 250-6000 $\mu S\ cm^{-1}$; Nachshon et al. 2014). The annual water budget is dominated by spring snowmelt, with groundwater inputs expected to be minimal in winter (D et al. 2009; Hayashi et al. 2016). St. Denis Creek is an ephemeral stream that feeds the catchments above ponds 1 and 90 in the SDNWA. The creek typically is dominated by spring snowmelt, but in recent years summer rainfall events have further fed the stream (Shook and Pomeroy 2012; Brannen et al. 2015; Mengistu and Spence 2016). When the creek flows, it flows from agricultural lands from the north into St. Denis (see Fig. 4-2). Year to year, the creek flow is variable, and the soil itself is frozen when the creek begins to flow.

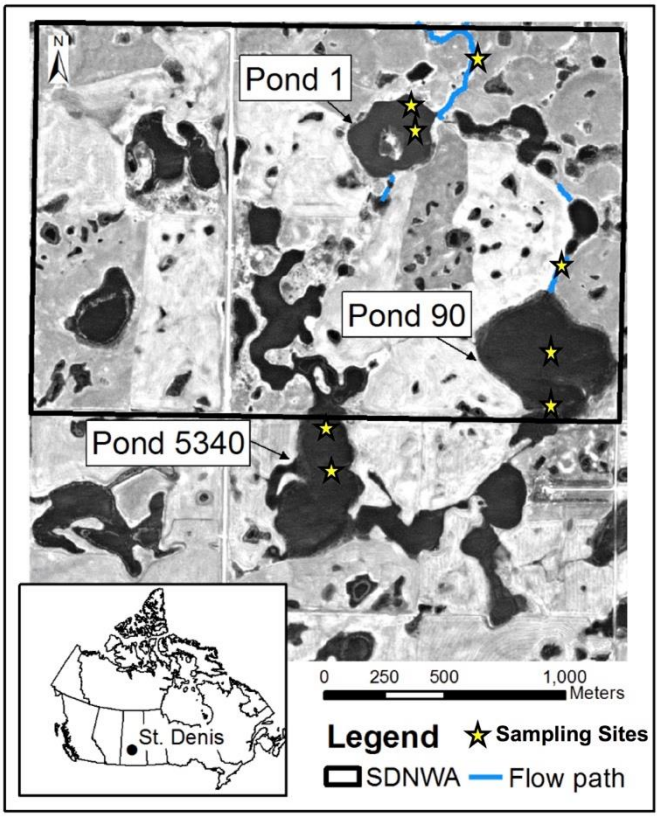

Figure 4-2 St. Denis National Wildlife Area showing the three ponds of interest, sampling locations and the flow paths. The ephemeral St Denis Creek flows from north to south and into the SDNWA: first flowing into Pond 1, then flowing into a series of semi-connected ponds ending in Pond 90. Pond 5340 is the terminal pond for this system and collects the fill and spill water in its basin.

4.3.2 Field sampling and lab processing

Water and gas samples were collected during the winters of 2014-2016 from the center of each of our study ponds via peristaltic pump at two depths. Water samples were collected near the surface or just under the ice (called surface water), typically between 0.5 and 1 m below the surface of the ice (as the ice thickness increases) and near the sediments (called bottom water), typically 0.5 m above the sediment surface. Sampling occurred monthly during the winter and the open water seasons and once or twice a week during the melt period. During winter, sampling was performed in a sheltered ice hut to protect water and gas samples from freezing. Nitrous

oxide (N_2O) was sampled via headspace equilibrations after overfilling a 1.2-L glass bottle with sample water (Cole et al. 1994). Ice depth was measured using a tape measure connected to a bar. Unsafe ice conditions in March and April necessitated a shift in sampling location to the near-shore area, where a hose was installed through the ice to allow shore-side sampling, referred to as the meltbox or melt location. In 2014, only one depth was collected in the near-shore (at approximately 0.5 m depth), and then in subsequent years, samples were collected from two depths (near the ice and near the sediment). Due to physical and personnel constraints, pond 1 was not sampled in the 2015/2016 year. To understand catchment inputs, St. Denis Creek water and snow samples were collected and analyzed for NH_4^+, NO_3^-, and SRP in the 2014/2015 winter from a Parshall flume and v-notch weir installed upstream of pond 1 and pond 90 (Fig. 4-2). Creek samples were collected as snow or flowing water until the creek ceased to flow during this season. Water and snow samples were collected regularly at the end of winter and more frequently while snowmelt runoff was flowing in the channel. Sediment samples for measurement of denitrification rates were collected using an Ekman grab (Standard Ekman Grab, Wildco ®, Yulee, FL) during winter, melt and open water periods in ponds 1 and 5340. In 2015, a Hobo Level Logger (Onset, Bourne, MA) was deployed in pond 90 to record changes in water level. Subsequently, the pressure was converted to depth measurements by correcting for barometric pressure and then applying a known offset.

In situ oxygen, temperature, pH, and specific conductance measurements were taken at the aforementioned sampling depths using the YSI 556 Multi Probe System (YSI Environmental, Yellow Springs, OH). Near the deepest part of each pond, time series of temperature, oxygen and conductivity were collected at two depths using the 600 OMS V2

sondes (YSI Environmental, Yellow Springs, OH) attached to MB-300 Data Buoy (NexSens Technology, Fairborn, OH) that was deployed under the ice in the early winter. The sensors were deployed such that the top sensors were near the ice (1.2-m below the ice surface at the beginning of the winter) and the bottom sensors were approximately 3-m below the ice (between 2-m and 0.5 m above the sediments). In the winter of 2013/2014, data were only recovered from the pond 1 buoy, in 2014/2015 all buoy data was recovered and in 2015/2016 data was recovered from ponds 1 and 90. In the winter of 2015/2016, the buoys were deployed before the onset of ice. Further, the spring of 2016 (March 23rd onward), the YSI malfunctioned and at ponds 90 and 5340 HOBO dissolved oxygen loggers (U26-001; Hoskins Scientific, Edmonton, Alberta) were installed at the collection site near shore (as above) at both sampling depths (0.5 m and 0.9 m for pond 90 and 0.8 m and 1.7 m for pond 5340). Water samples, during that same period, were measured for specific conductance and pH back in the laboratory, the same day, using the Orion Star A329 Multiparameter meter with the Orion 013010 MD conductivity cell and the Orion 8107 UWMMD Ross Ultra pH/ATC Triode (Thermo Fisher Scientific, Waltham, MA). Air temperature data collected at SDNWA by Environment Canada was used to verify warming associated with spring-melt and can be found at the Changing Cold Regions Network (http://giws.usask.ca/meta/Metadata_SDNWA.html; last accessed on July 5, 2018). Images of snow and ice melt were recorded via time-lapse cameras at the ponds to inform our understanding of the timing of events.

Water samples were filtered through pre-rinsed 0.2 μm polycarbonate filters; (A.M.D. Manufacturing, Mississauga, Ontario) then analyzed for nutrients using standard methods. Specifically, water samples were analyzed for nitrate (NO_3^--N and NO_2-N EPA method 353.2, hereafter referred to as NO_3^-), NH_4^+ (EPA 350.1) and soluble reactive phosphorus (EPA 365.1)

using the SmartChem 170 autoanalyzer (Westco Scientific Instruments, Inc., Brookfield, CT). Chlorophyll (uncorrected for pheophytin) was analyzed as the fraction retained on glass fiber filters (0.7 μm filters, using Wintermans and de Mots 1965 method). Water isotope samples were analyzed for their hydrogen and oxygen stable isotopic compositions by Off Axis Integrated Cavity Output Spectroscopy (OA-AICOS) using a Los Gatos Research DLT-100 laser spectrometer. We used two calibrated reference waters (INV1 δ2H = -217.7, δ18O = -28.5 and ROD3 δ2H = -3.9, δ18O = -1.0 per mil, respectively) to normalize raw delta values to the VSMOW–SLAP scale. To minimize memory effects, samples and reference waters were injected 9 times and the last 5 measurements were averaged to obtain the final raw delta values. Precisions as determined by replicate analyses of samples and reference waters were ± 1 and 0.1 per mil for hydrogen and oxygen, respectively. Headspace N_2O gas samples were analyzed, in duplicate, using the Scion 456 Gas Chromatograph (Bruker Ltd.). A micro-electron capture detector (ECD) was used to measure N_2O. To calculate the appropriate salinity for solubility corrections, filtered water samples were analyzed for ions using Inductively Coupled Plasma – Optical Emission Spectrometry, Department of Geology, University of Saskatchewan. These corrected salinity values were then used to calculate ionic salinity (as per Pawlowicz 2008) due to the dominance of calcium, magnesium and sulfate ions. The appropriate salinity was used in concentration calculations in standard solubility equations for N_2O (Weiss and Price 1980).

4.3.3 Measurement of biogeochemical rates

To characterize the importance of nitrogen uptake through late winter, melt, and the early open water period, we measured ^{15}N uptake of dissolved inorganic nitrogen from ponds 90

and 5340 as described in Gu et al. 1997. This work was completed in 2016, using water samples collected as previously described. Ammonium uptake experiments were conducted in winter, melt, and shortly following ice-out. Nitrate uptake experiments were conducted in melt only due to low winter NO_3^- concentrations at the time of sampling and shortly after the ice was gone. Water samples for uptake experiments were obtained as previously described and protected from light prior to return to the lab. Once in the lab, sample water was transferred into Winkler bottles, enriched with ^{15}N-enriched NO_3^- or NH_4^+ (potassium nitrate-^{15}N Lot number: MBBB1915V; NH_4^+ -^{15}N chloride Lot number MBBBB2704V; both at 98% atom ^{15}N; Sigma Aldrich, St. Louis, MO) to approximately 10% of *in situ* concentrations and incubated.

Light measurements were obtained from under the ice using an arm and a LI-COR underwater sensor and data logger (UW 8740 underwater sensor, LI-1400 data logger; LI-COR, Inc. Lincoln, NE) and used to estimate winter light conditions (approximately 30 μmol $m^{-2}s^{-1}$). Experiments were performed under low light conditions (~30 μmol $m^{-2}s^{-1}$) and dark conditions. Incubations were performed for 4 and 24 hours because of concern about measurability of low uptake rates. After the incubation period was over, the water was filtered onto precombusted GF-F (0.7 μm) filters in the dark. The filters were dried, transferred to tin capsules and analyzed at the University of California, Davis, Stable Isotope Laboratory via Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK; http://stableisotopefacility.ucdavis.edu/). Uptake rates were calculated as per Kumar et al. (2008). The ^{15}N particulate mass (P^*) on the filters was converted to uptake rate using percent increase in ^{15}N over the incubation period (ΔI_p), time of incubation (T^*), the natural abundance atom-% ^{15}N (I_o), the tracer spike atom-% ^{15}N (I_r), ambient N (S_a), and added ^{15}N (S_t):

$$uptake\ rate\ (\mu mol\ N\ L^{-1}\ h^{-1}) = (P^*\ \times\ \Delta I_p)/\left(T^* \times \frac{\{I_o \times S_a + I_r \times S_t\}}{S_a + S_t} - I_o\right) \quad \text{..... (4.1)}$$

The natural abundance of ^{15}N for these ponds was assessed by Dr. Rebecca North (unpublished data for particulate ^{15}N). The rate generated is in units of μg N L^{-1} h^{-1}. The limit of quantification (LOQ) for dissolved inorganic nitrogen uptake rates was assessed for each sample (average LOQ: 3.8 x 10^{-8} μg N L^{-1} h^{-1}). The LOQ was calculated based of the method detection limit (MDL) in ^{15}N atom-% of the lab's reference standards (MDL = standard deviation ^{15}N at-% × Student t Distribution quantile for n degrees of freedom at a 95% confidence interval). None of the observed nitrogen uptake rates were lower than their respective LOQ.

Denitrification and nitrification experiments were performed as per Chapter 2 and 3, respectively. Nitrification rates were measured in the melt period of 2016 at ponds 90 and 5340 through $^{15}NH_4^+$ enrichment incubation and ^{15}N recovery following slightly modified (for freshwater) protocols from Carini and Joye 2008 and Sigman et al. 1997. Denitrification rates in winter and melt of 2014 and 2015 were measured in ponds 1 and 5340 following acetylene block method of Smith et al. 1978. Experimental conditions for denitrification included NO_3^- amendment as published in Cavaliere and Baulch (2018). Nitrate was added to sediment-water slurries at a concentration of 100 mg NO_3^--N per kg of sediment (Cavaliere and Baulch 2018).

4.3.4 Statistical analysis

All data were processed using the R statistical package (R Core Team 2018). Chemistry data were not normal and required the use of non-parametric tests. Conductivity increases during winter (Pieters and Lawrence 2009) and will begin to decline in spring-melt. To define

these seasons we used segmented linear permutation models to identify when changes in conductivity occurred (‘lmp’ function; lmPerm package; Wheeler and Torchiano 2016b & ‘segmented function’; segmented package; Vito and Muggeo 2003). The ‘segmented’ function identifies the timing of a breakpoint (in this case using the variable days). This point is when the slope of the linear model begins to change ('segmented' function; segmented package; Vito and Muggeo 2003). Data were then split according to these breakpoints, which were used to define the split between increasing conductivity in the winter period and declining conductivity in the melt period, while the open-water period is defined as after complete ice melt. Repeated measures permutation ANOVAs were then used to analyze differences among periods (winter, melt, open water) and years for the different nutrients ('aovp', lmPerm Package; Wheeler and Torchiano 2016b), based on the breakpoints identified for the winter and melt periods. Permutation tests perform a series of t-tests without regard for the original groupings of the data; creating a distribution of t-statistics which, when compared to the original grouping, we can determine if the data fall inside or outside the distribution and designated alpha value (0.05) (Kabacoff 2011; Wheeler and Torchiano 2016b). For the repeated measures ANOVA, individual ponds were analyzed separately, with years as replicates – controlling for autocorrelation of days through repeated measures. This approach had similar results compared to an analysis of years with the ponds as replicates (not shown). The repeated measures ANOVA formula was as follows:

Analyte ~ period*year*days since ice on + Error(pond/days since ice on)…….. (4.2)

This formula compares period (between subject), years (between subject) and days since ice on (within subject) is a repeated measure, while pond is the subject of the test. The interactions

between period, year and days since ice on will give an indication of interacting impacts (between season and year, period and days since ice on, year and days since ice on) on the individual significance of that particular variable. If an interaction is significant, than that would indicate that interpretation of the interacting explanatory variables on the response variable would be complicated. The repeated measures term (Error) accounts for the within repeated measures error (days since ice on) within the pond (subject) effect. If the ANOVA was significant for either period or year, a post hoc pairwise permutation test was used to look for specific pairwise differences ('pairwisePermutationTest' function; rcompanion package; Mangiafico 2017). The posthoc tests tested for differences among the three years (2013/2014, 2014/2015, 2015/2016) and among the three periods (winter, melt, and open water).

The Kendall partial correlation trend analysis, a non-parametric ranked correlation, was used to look at trends within seasons among ponds, using years as replicates ('pcor.test' function; ppcor package; Kim 2015; R Core Team 2016). To compare differences between two groups when data are not normal, we used the non-parametric Wilcoxon-Mann-Whitney test ('wilcox_test' function; coin package; Hothorn et al. 2008b). The wilcox_test was used to compare periodic (winter vs melt) pelagic and benthic sites, and assess differences between nutrient concentrations in the pond and creek, within periods. This test was also used to compare uptake rates for NH_4^+ and NO_3^-, light and dark uptake rates and assess if denitrification rates were NO_3^--limited ('wilcox_test' function; coin package; Hothorn et al. 2008b). A permutation, one-way ANOVA ('aovp' function, lmPerm package; similar to the above repeated measure ANOVA) was used to look at period differences in denitrification rates. In addition, a principal components analysis (PCA) was used to assess, in a non-parametric, qualitative way, the associations among variables ('prcomp' function; stats

package; R Core Team 2016). Here, we used PCA to compare surface and bottom water associations in winter and melt. A PCA can show how different variables are related to each other and along a specific component. The closer in promixity two variables are, the more closely related they are, and along an axis, where those variables relate to a specific component. The two major components are plotted, and along those axes, often a dominant variable will control the variability explained by that component.

To compare period (winter, melt, open water) impacts on nitrogen uptake rates, a linear mixed effects model was used ('lme' function; lmerTest package; Kuznetsova et al. 2017). This approach has more flexibility when variances are heteroscedastic, while still controlling for repeated measures effect of sampling the same ponds over time. The lme model assessed for differences among periods for light uptake of NH_4^+ over time. The rate data were not normal and transformed using the BoxCox function ('boxcox' function; MASS package; Venables and Ripley 2002). The non-normality of this data was improved but remained skewed towards low values. Despite this, the residuals from this model were normal and centered around a mean of zero, suggesting non-normality had a minimal impact on the lme model. To compare within period differences, a posthoc test of multiple comparison of means by Tukey contrasts was used on the model ('glht' function; multcomp package; Hothorn et al. 2008a).

4.4 Results

4.4.1 Seasonality

Ice thickness increased through much of winter (to approximately day 90-120; across ponds and years), with concurrent increases in surface conductivity (Fig. 4-3). Ice typically reached a maximum thickness of ~80 cm, but occasionally reached thicknesses of 1 meter (Appendix B

Fig. B.1). Over the winter period, conductivity increased on average 25% (range: 8-48%) from the onset of the ice cover period to the time of maximum conductivity. In general, conductivity shows a linear increase until mid-winter ('lmp' function: $P < 0.05$; median adjusted R^2: 0.35), when surface conductivity stabilizes, and then starts to decline – this inflection point or breakpoint has been identified for each of the three winters through the breakpoint analysis ('segmented' function; Fig. 4-3).

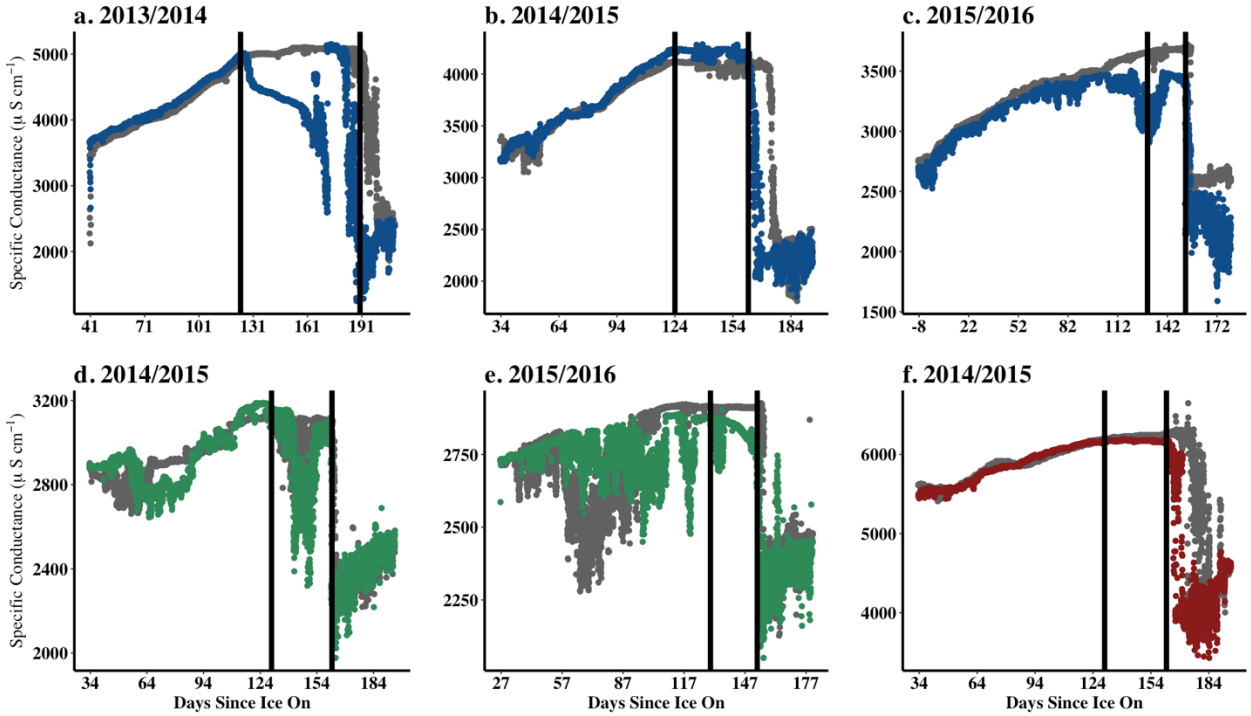

Figure 4-3 Time series for conductivity over the three years; two sensors were used to collect this data, in the water column there was a sensor near the ice (1.2-m below the ice surface at the start of winter) and one approximately 3-m below the ice surface. Pond 1 is represented in a-c, pond 90 d and e, and pond 5340 in f. The individual colors represent the different ponds: pond 1 is blue, pond 90 is green and pond 5340 is red, while the gray in all ponds is the bottom sensor. The first line is the day identified by the breakpoint analysis as the onset of melt, while the second line is the day the ice was completely off the ponds. Note missing series are due to instrument malfunction.

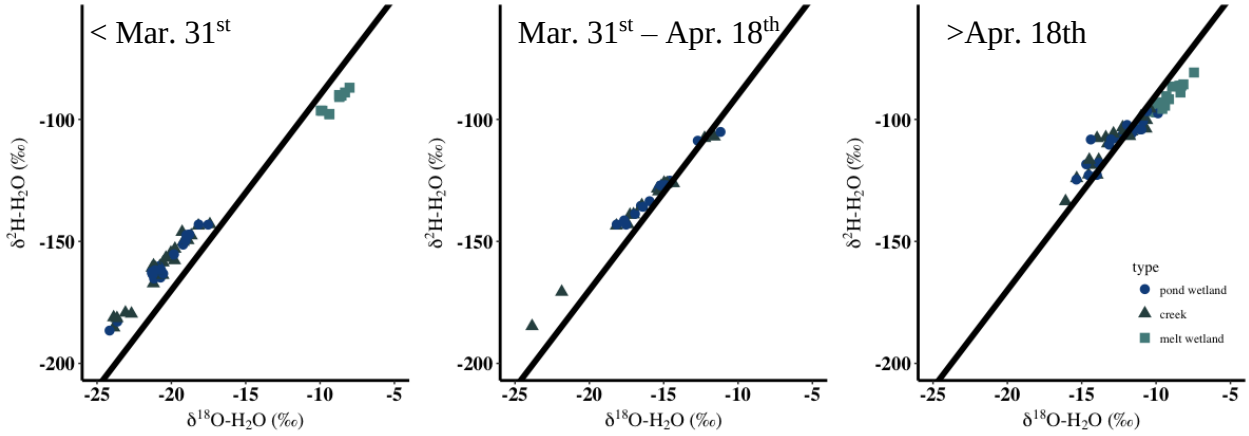

Figure 4-4 Stable water isotopes of hydrogen and oxygen. The line is the global meteoric water line (Rozanski et al. 1993). The type of sites include pond wetland (winter sampling location), melt wetland (spring-melt sampling site on ponds) and creek (above both ponds 1 and 90).

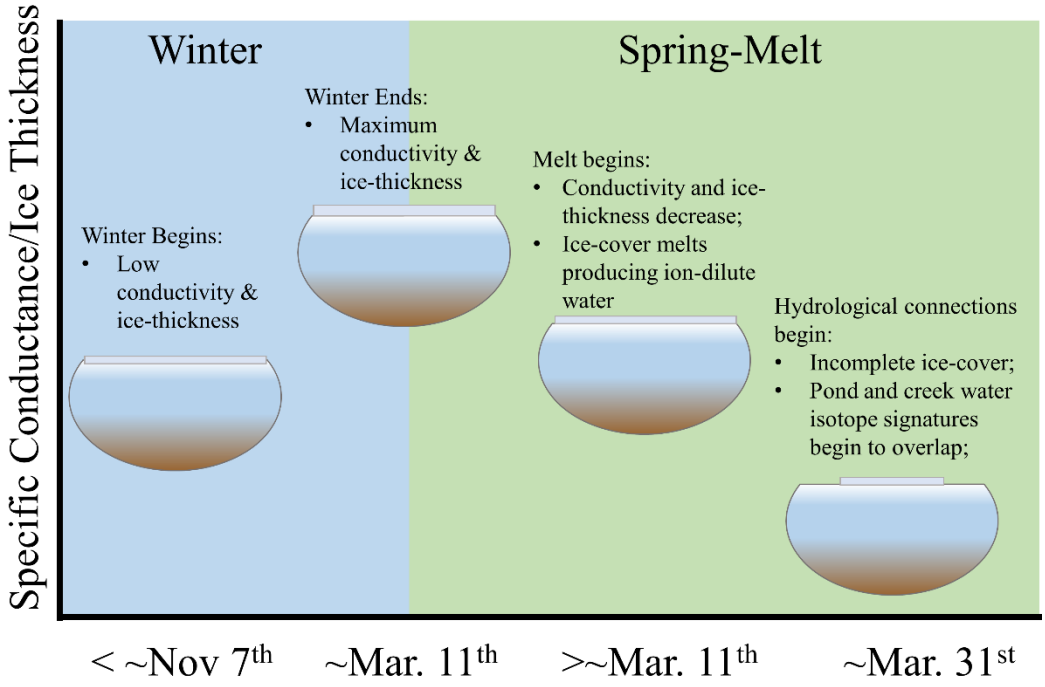

Figure 4-5 Conceptual diagram of conductivity and isotope signature shifts during the melt period of the 2014/2015 winter. The relative specific conductance and ice-thickness is indicated by the pond symbols. The conductivity and ice increased until March 11, 2015, when conductivity reached its peak, after which, internal melting of the ice diluted the conductivity indicating melt was active at this point (Fig. 4-3). Beginning on March 31st, creek and pond water isotopes begin to overlap (Fig. 4-4), indicating that there was mixing and connections were made between the creek and pond.

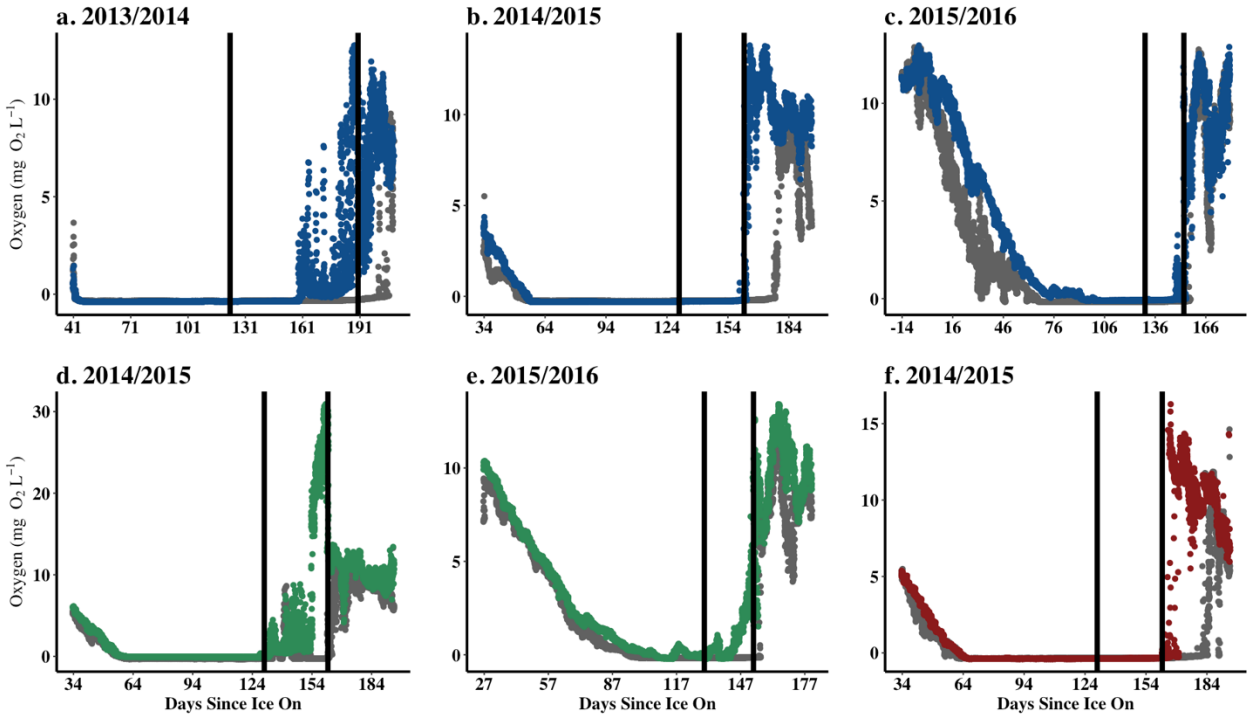

Figure 4-6 Time series for measured dissolved oxygen over the three years; two sensors were used to collect this data, in the water column there was a sensor near the top (1.2m from the bottom of the ice) and one approximately 3-m below the ice surface. Pond 1 is represented in a-c, pond 90 d and e, and pond 5340 in f. The individual colors represent the different ponds: pond 1 is blue, pond 90 is green and pond 5340 is red, while the gray in all ponds is the bottom sensor (data sometimes obscured by the dots from the upper sensor). The first line is the day identified by the breakpoint analysis as the onset of melt, while the second line is the day the ice was completely off the ponds. The instrument detection limit was 0.1 mg L^{-1}. Note missing series are due to instrument malfunction.

The winter of 2013/2014 was a long winter, with the ice-cover period lasting 190 days. Surface conductivity increased from 3667 µS cm^{-1} in pond 1 (single buoy data for 2013/2014) at the start of winter to a maximum of 5148 µS cm^{-1}, reaching an ice thickness of nearly a meter in this pond where maximum water depth was only 3.5 m. Breakpoint analysis identified an inflection point in conductivity around 124 days after ice formation (March 5, 2014; Figure 4-3a). Surface night and day air temperatures did not begin to rise consistently above 0°C until four days later, at the same time, day length exceeded 11 hours, and the solar angle is high, contributing to melting (Global Instituted for Water Security 2018; Air

temperature data). In the two subsequent years, the inflection point in conductivity was later and co-occurred with the period when the night and day air temperature consistently exceeded 0°C and day lengths were longer. During the winter of 2014/2015, this inflection point occurred around March 11th, 2015 (mean for the three ponds: 124 days and range of 118 to 127 days after ice formation; Fig. 4-3b,d,f). In the subsequent year, the mean inflection point in conductivity beginning around March 27th, 2016 (mean: 130 days and range of 129 to 131 days after ice formation; Fig. 4-3c,e), which followed warming air temperatures that started around March 19th, 2016 (Global Instituted for Water Security 2018; Air temperature data). It is important to note that within a year, the breakpoint in conductivity varied little across ponds (≤9 days). Across years, this breakpoint varied by 13 days, but consistently occurred within ~2 weeks of the vernal equinox (March 20th). Pond 90 appears to show greater and more frequent oscillations in conductivity than the other two ponds (Fig. 4-3).

Water isotope data were used to assess water sources and compare to conductivity-based measurements of the onset of spring-melt. These data collected during the winter of 2014/2015 in the ponds, and the creek (Fig. 4-4). The pond isotopic signatures are typically enriched (comparatively) and evaporated, while the creek ponds are depleted and more typical of snow (Bam et al. *Under Review*). The water isotope signatures show separation of the pond and creek isotopic signatures during winter. The $\delta^{18}O$ and $\delta^{2}H$-H_2O values for the pond and creek start to overlap around March 31st, 2015. This is a later than the March 11th date identified by the breakpoint in conductivity (Figs. 4-3, 4-4 & 4-5). The different dates indicated by the breakpoint analysis of conductivity and isotope signature overlap are likely a result of different processes. The earlier conductivity shift is likely due to the importance of incoming fresh water from melting of overlying ice early in the melt process. This occurs prior to snowmelt inflows from the

watershed (Figs. 4-4, 4-5, and 4-7). As snowmelt starts, isotope signatures shift likely as a response to increased connectivity and water exchange among the ponds and ephemeral creek due to mixing of the snow and ice melt water (Fig. 4-4). We subsequently define the different periods of winter using these conductivity-derived inflection point, applying the average date from across the ponds when sensor deployment was successful. The winter period is defined as before the breakpoint and melt is defined as the period between the breakpoint day and complete ice-off. Open water is subsequent to melt. All other parameters are grouped into seasons derived from these seasonal thresholds.

Table 4-1 Timetable of ice formation, average onset of hypoxia (<2 mg L^{-1} O_2), hypoxia ending (>2 mg L^{-1} O_2) and melt and complete ice off (days are in days since ice on). Dates are average across ponds for which there were successful sensor deployments. The start of ice melt was determined, as outlined above, through use of conductivity-derived inflection points. Completion of ice melt was monitored using time-lapse photography and field-based observation.

Year	Ice Forms	Hypoxia Begins	Hypoxia Ends	Ice Melt Begins	Ice Melt Complete
2013/2014	Nov. 1st, 2013	Prior to Dec. 12, 2013 (41 days)	Apr. 7th, 2014 (157 days)	Mar. 5th, 2014 (124 days)	May 10th, 2014 (190 days)
2014/2015	Nov. 7th, 2014	Dec. 22nd-25th, 2014 (45-48 days)	Mar. 18th-Apr. 19th, 2015 (132-164 days)	Mar. 11th, 2015 (124 days)	Apr. 18th, 2015 (162 days)
2015/2016	Nov. 18th, 2015	Jan 9th-23rd, 2016 (52-66 days)	Apr. 12th, 2016 (146 days)	Mar. 27th, 2016 (130 days)	Apr. 19th, 2016 (153 days)

4.4.2 Oxygen dynamics

Oxygen concentrations declined over winter in all ponds and all years, with a long period of winter hypoxia exceeding 2 months (average days of hypoxia: 98.6 days; range: 80-116 days; Table 4-1). In the winter of 2013/2014 the onset of hypoxia was prior to Dec 12, 2013 (prior to instrument deployment; within 41 days of ice formation; Table 4-1 & Fig. 4-6). In the winter of 2014/2015, oxygen decline started immediately after ice formation and the

ponds were anoxic after 45 days (Fig. 4-6a). In the winter of 2015/2016, we were able to capture more of the oxygen decline period, and unique to that year, the oxygen persisted well into the ice cover period. Late winter changes in oxygen were dramatic, and the end of hypoxia consistently preceded ice-out. Pond 90 showed an increase in oxygen concentrations above 2 mg L^{-1} at the onset of melt, rising to in excess of 30 mg L^{-1} in spring of 2015 prior to complete ice off (Fig. 4-6d). The oxygen stayed above 20 mg L^{-1} for several days during this period and while ice was visible on the pond, it was quite thin (Fig. 4-7).

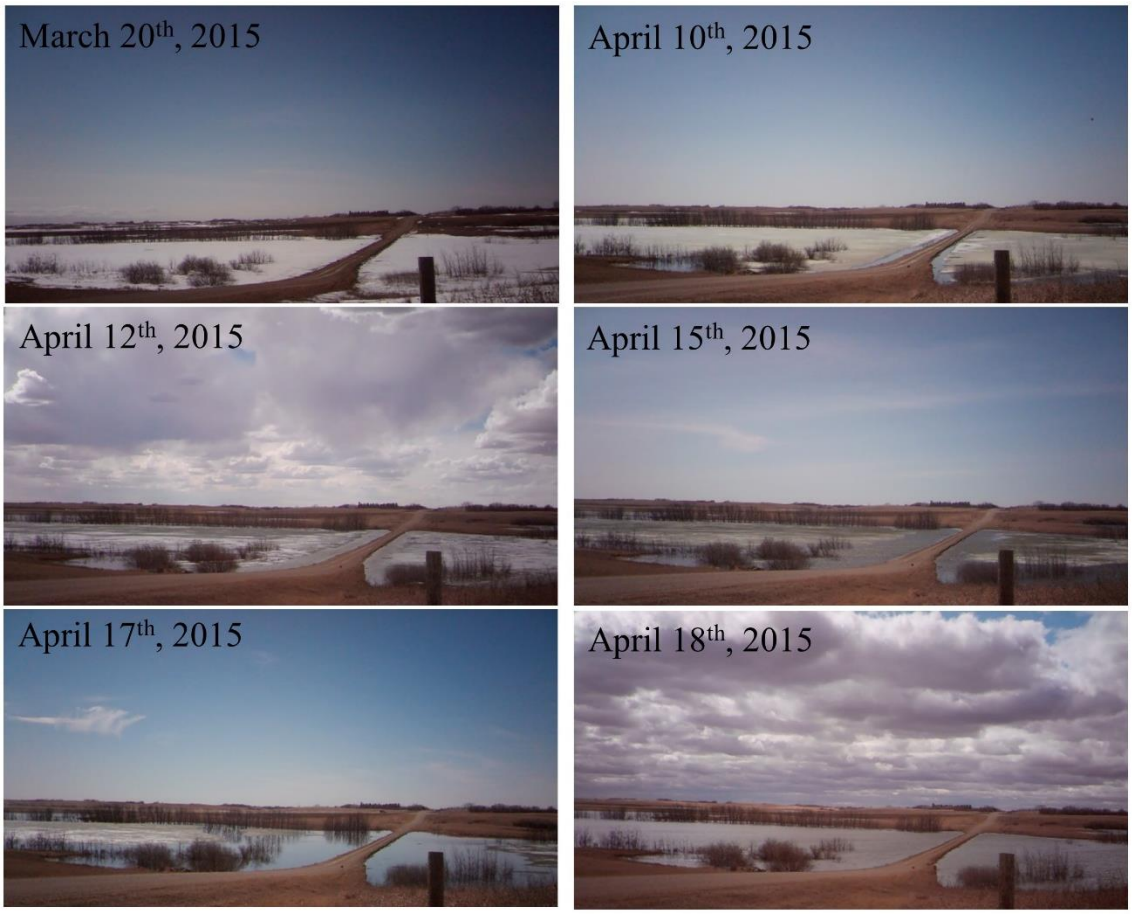

Figure 4-7 Time lapse photographs of pond 5340 at 3PM. Earlier snow covered conditions are represented on March 20th, while low snow and melting ice conditions are visible in subsequent photographs. On April 18, 2015 the ice was completely melted. Similar trends were observed in the field for both ponds 1 and 90.

In 2013/2014 oxygen concentrations in Pond 1 also increased prior to ice off, but only reached concentrations of 13 mg/L (Fig. 4-6a). In both the winters of 2014/2015 and 2015/2016, pond 1 showed a relatively stable oxygen and conductivity time series through melt as compared to the previous year when conductivity was observed to rapidly change after the inflection point (Fig. 4-3b,c & Fig. 4-6b,c). Similarly, the conductivity and oxygen in pond 5340 in the winter and spring 2014/2015 remained fairly constant until the ice was completely gone (Figs. 4-3f &4-6f). The oxygen concentrations measured by the hand-held YSI during the winter and melt period followed the above trends. During the melt period the sampling location was near shore and the YSI data show evidence of earlier oxygen infiltration, particularly in pond 5340 where oxygen concentrations began to rise prior to complete ice-off. The oxygen concentrations from the buoy sensors, as shown in Fig. 4-6d, show that pond 90 oxygen concentrations reached 30 mg L^{-1}; the sensor itself has a range of 0.1 to 50 mg L^{-1}. The handheld YSI reflected this increase, but likely due to the local influence of the sediments only reached oxygen concentrations of 14.5 mg L^{-1}.

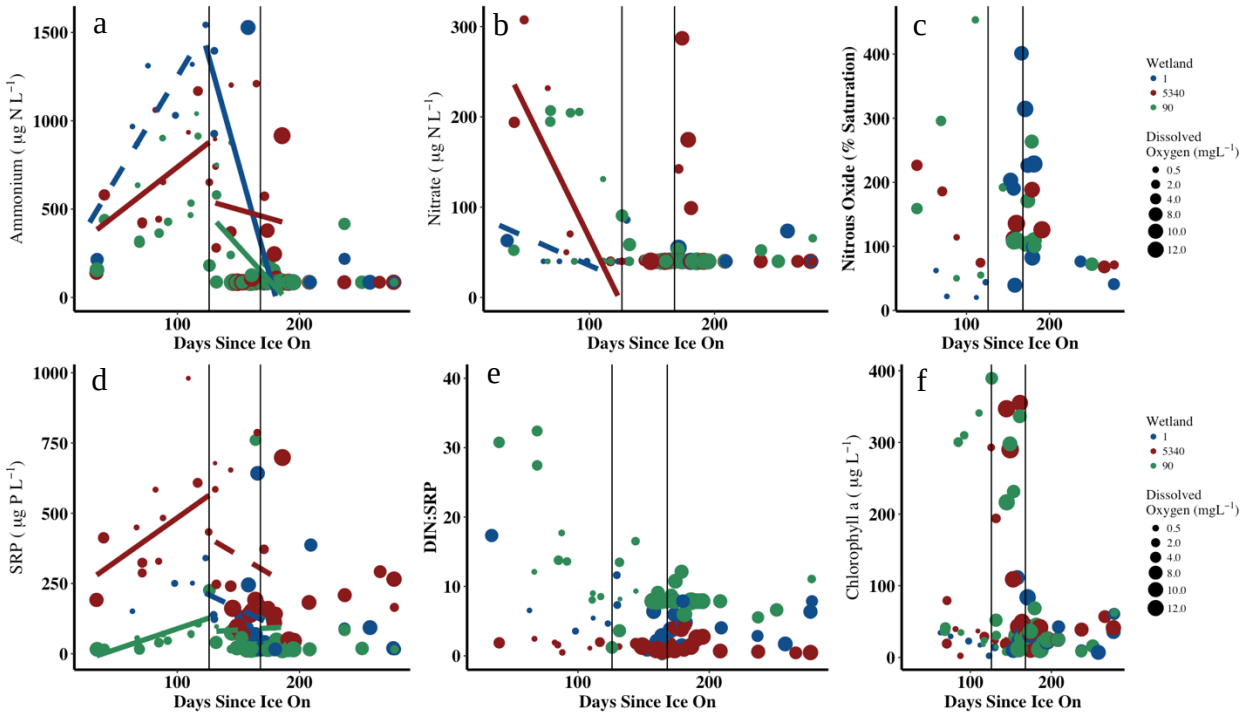

Figure 4-8 Time series of surface NH_4^+, NO_3^-, N_2O, SRP, the ratio of DIN:SRP and chlorophyll over the three years and three ponds. Note the vertical lines are the average onset of melt (first line) and the average ice-off date (second line). The fit lines are indicative of significant seasonal trends for that specific pond ('pcor.test' function; Kendall correlation, solid lines are significant P <0.05; dotted lines are marginally significant P <0.1). The ratio of DIN to SRP plot has had an extreme outlier removed (N:P = 101). The increases in NH_4^+ and SRP are associated with lower dissolved oxygen concentrations (smaller size bubbles) in winter, while chlorophyll is more commonly associated with higher concentrations of dissolved oxygen in the melt period (larger size bubbles).

4.4.3 Changes in pond chemistry

A comparison of mid-pond, pelagic (winter sampling location) and near-shore, benthic (melt sampling location) samples revealed that no significant differences between the pelagic and the nearshore environment, with the exception of NH_4^+ concentrations in the bottom waters. The near-sediment pelagic environment had higher NH_4^+ concentrations compared to the near-sediment, near-shore benthic environment (wilcox_test; P = 0.01, 14 DF: mean pelagic: 3073.6 µg N L^{-1}; mean benthic: 546.9 µg N L^{-1}). For both the surface and bottom water concentrations, NO_3^-, SRP and chlorophyll were all quite similar, with no statistical difference found for any combination of surface or bottom water (wilcox_test; P >0.05). Nitrate concentrations between

the pelagic and the nearshore environment were similar in range and mean (mean pelagic: 54 μg N L^{-1}; mean benthic: 78 μg N L^{-1}). SRP concentrations were similar between the two sites as well, despite a slightly higher mean in the pelagic environment (mean pelagic: 505 μg P L^{-1}; mean benthic: 308 μg P L^{-1}). Chlorophyll concentrations were similar between the two sites (mean pelagic: 124 μg L^{-1}; mean benthic: 97 μg L^{-1}).

To test for differences in solute concentrations across seasons, an ANOVA (aovp) with repeated measures was used. To determine if solute concentrations changed within seasons, a partial correlation using Kendall's tau (pcor.test) was used (correlation between analyte and days since ice on, given year; tau indicates strength and direction of correlation). Surface water NH_4^+ concentrations changed between winter, melt and the ice-free season (aovp; P <0.001; 97 DF, Fig. 4-8). In Fig. 4-8a (as for Figs. 4-6 a-f), surface water NH_4^+ concentrations are shown over the sampling year (days since ice on) where each season is delineated by vertical lines and within season significant trends are represented by lines specific to each pond. Surface water NH_4^+ concentrations in winter (mean: 657 μg N L^{-1}) were consistently higher than melt (mean: 414 μg N L^{-1}) and open-water (mean: 102 μg N L^{-1}) concentrations (pairwisePermutationTest; P <0.01; Fig. 4-8). There was a significant upwards trend in surface water NH_4^+ concentrations within winter for pond 5340 (pcor.test; tau = 0.5; P = 0.02) and a marginally significant trend for pond 1 (pcor.test; tau = 0.7; P = 0.08), while pond 90 did not exhibit a significant trend (pcor.test; P = 0.1). Kendall's tau gives an estimate of the strength and direction of the correlation.

Due to evidence of variation among sampling sites in bottom-water NH_4^+, results of comparisons across seasons should be interpreted with caution. These comparisons suggest there are no significant seasonal differences in bottom water NH_4^+ (aovp; P = 0.6; 70 DF).

However, within winter, bottom concentrations of NH_4^+ increased significantly in pond 5340 (pcor.test; tau = 0.45; P = 0.03) and marginally significantly in pond 1 (pcor.test; tau = 0.7; P = 0.07), while no significant trends were observed for pond 90 (pcor.test; tau = 0.1; P = 0.6). Following these upward trends in winter NH_4^+ concentrations in surface water (and in some ponds in the bottom waters), during the melt period NH_4^+ concentrations declined in the surface and bottom waters (Kendall's tau estimates between -0.2 and -0.7; pcor.test; Surface: pond 1: P = 0.004; pond 90 P = 0.007; pond 5340 P = 0.049; Bottom: pond 1: P = 0.6; pond 90 P = 0.03; pond 5340 P = 0.046).

Surface water NO_3^- concentrations showed no apparent differences across seasons (aovp; P = 0.23; 85 DF; Fig. 4-8); however, within the winter period NO_3^- concentrations in both the surface and bottom waters decreased significantly in pond 5340 (pcor.test; surface: tau = -0.7; P = 0.003; bottom: tau = -0.6; P = 0.01). Within the remaining two ponds, there was weaker evidence of change in NO_3^- through winter. Nitrate appeared to decrease in the surface waters of pond 1 during winter (pcor.test; tau = -0.8; P = 0.06), and in the bottom waters of pond 90 (pcor.test; tau = -0.4; P = 0.1), although these trends are marginally significant, reflecting potentially slightly higher NO_3^- concentrations early in the winter. Only low concentrations were observed during the melt period (< 56 μg NO_3^- L^{-1}; Fig. 4-8b).

Nitrous oxide was highly dynamic over the sampling period (Fig. 4-8c), ranging from well below saturation to >400% saturation. During winter, N_2O was typically oversaturated (surface mean: 143%, range: 20-454%; bottom mean: 154%, range: 50-296%) while during the open water season three-quarters of measured N_2O concentrations were below saturation (surface mean: 76%, range: 27-126%; bottom mean: 29%, range: 20-401%). There were no significant differences among seasons (aovp; P = 0.13; 26 DF) or trends within seasons (pcor.test; P >0.05).

Concentrations of SRP were higher in winter (pairwisePermutationTest; $P < 0.001$) and melt (pairwise permutation test; $P = 0.03$) periods than the open water period (aovp; $P < 0.001$; 99 DF; Fig. 4-8). Within winter, soluble reactive phosphorus concentrations increased significantly in the surface waters in ponds 90 (pcor.test; tau = 0.7; $P < 0.001$) and 5340 (pcor.test; tau = 0.46; $P = 0.03$) and in the bottom waters of pond 90 (pcor.test; tau = 0.44; $P = 0.04$). SRP concentrations in pond 1 did not increase or decrease significantly in surface or bottom waters during winter (pcor.test; surface: $P = 0.1$; bottom: $P = 0.2$), nor did SRP in the bottom waters of pond 5340 (pcor.test; $P = 0.9$). In all ponds, surface water SRP concentrations increased, on average, from 115 μg P L^{-1} in early winter (first 60 days) to 323 μg P L^{-1} in the last month (30 days), while the bottom waters increased even more – four-fold over that same period (on average, from 131 to 533 μg P L^{-1}). During melt there were significant declines in SRP concentrations at both depths in pond 5340 (pcor.test; surface: tau = -0.44; $P = 0.03$; bottom: tau = -0.61; $P = 0.04$). Moderate declines were observed in surface SRP concentrations over the melt period in ponds 1 (pcor.test; tau = -0.4; $P = 0.08$) and 90 (pcor.test; tau = -0.36; $P = 0.07$), but trends in the bottom waters were not observed (pcor.test; $P > 0.05$). Changes in SRP concentrations during melt were followed by relatively stable concentrations in the open-water period (Fig. 4-8d). The ratio of DIN to SRP appears to shift at the onset of winter, however, changes do not appear to be specific to the phase of winter as defined here (Fig. 4-8e).

Differences in surface chlorophyll concentrations were observed across the winter, melt and open water periods (Permutations repeated measures ANOVA; Surface: $P = 0.001$; 100 DF; Bottom: $P = 0.067$; 74 DF; Fig. 4-8f). Winter chlorophyll concentrations appeared to be higher, while both the melt and open water periods had greater variability (Fig. 4-8f).

However, it is important to note that the posthoc tests revealed no specific pairwise seasonal differences (pairwise permutation test; $P > 0.05$). There were no seasonal trends in surface or bottom chlorophyll concentrations (pcor.test; $P > 0.05$).

The principal components analyses in Fig. 4-9 shows the strength of relationships between variables along the principal components axes PC1 and PC2. The PCA for surface winter variables vary along the PC2 axis which is associated with oxygen concentrations; while the PC1 axis varies along a temperature gradient. The bottom water PCA in winter is similarly distributed, with dominance of oxygen on PC2 while temperature along the PC1 axis. The PCA for surface water in winter shows that conductivity and SRP are strongly related to each other and along the PC1 axis (Fig. 4-9a) with NO_3^- and dissolved oxygen related along the PC2 axis. Conversely, in the bottom waters (Fig. 4-9b), nutrients (SRP and NH_4^+) are more strongly related to each other; they are also linked to the colder temperatures axis of PC1 and do not show a strong relationship to dissolved oxygen. During melt, NH_4^+ and SRP continue to show a close association in surface and bottom waters. SRP and NH_4^+ are more strongly dominated by low oxygen (along the PC1 axis) in the bottom waters during winter and melt (Figs. 4-9b,d) compared to their location in the surface waters (Figs. 4-9a,c). In winter, N_2O saturation in bottom and surface waters is linked to temperature, with an association with NO_3^- apparent in the bottom waters. In melt, N_2O is linked to NO_3^- and chlorophyll concentrations. During the spring-melt, the surface waters lose the association along the oxygen gradient, and are instead dominated by days (as related to onset of ice-cover). The bottom water variables, during melt, are strongly controlled by a temperature gradient along the PC1 axis (Figs 4-9c,d).

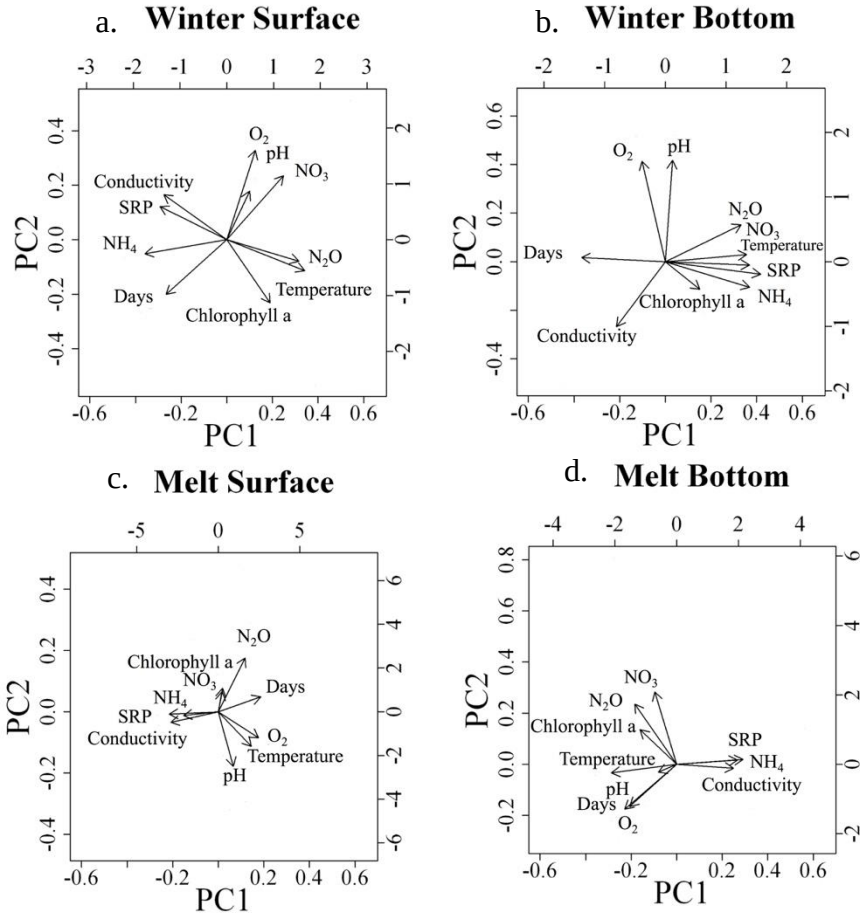

Figure 4-9 Principal component analysis of all ponds in winter and melt showing surface and bottom associations among nutrient concentrations and water chemistry.

4.4.4 Creek dynamics

During the melt and open water periods of 2014/2015, pond and creek samples were compared (Fig. 4-10). During spring- melt, NH_4^+ concentrations were significantly higher in the ponds (mean: 311 µg N L^{-1}) compared to the creek concentrations (mean: 164 µg N L^{-1}; wilcox_test; P = 0.03, 72 DF). Nitrate concentrations in the creek (mean 206 µg N L^{-1}) were nearly five-fold higher than concentrations in the ponds during melt (mean 42 µg N L^{-1}; wilcox_test; P < 0.001; 74 DF), while creek concentrations had a higher maximum SRP (maximum: 1363, mean: 377 µg P L^{-1}) compared to concentrations in the ponds (maximum

716; mean 199 µg P L^{-1}). However, the variability in SRP was large, and no significant differences were found between the creek and the pond (wilcox_test: P = 0.4; 72 DF).

After the ice melt was complete, concentrations of NO_3^- (wilcox_test: P = 0.54; 43), NH_4^+ (wilcox_test: P = 0.22; 46 DF) and SRP (wilcox_test: P = 0.12; 42 DF) remained similar between the creek and pond (no significant difference between sites; wilcox_test; P >0.05) but both systems seem to show a decline in NH_4^+, NO_3^- and SRP concentrations (Fig. 4-10).

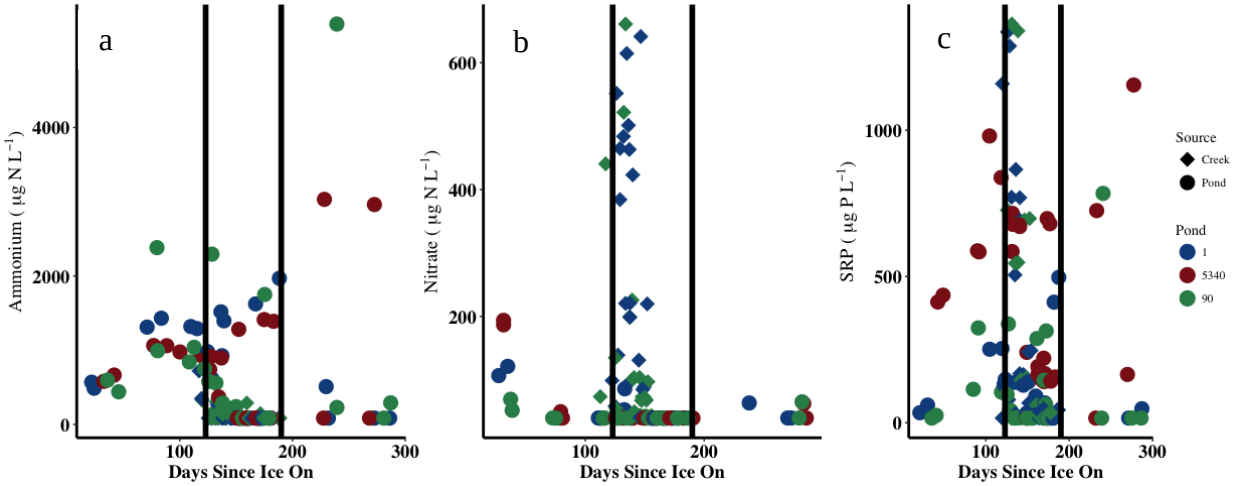

Figure 4-10 A comparison of the creek and pond chemistry during the 2014/2015 year. NH_4^+, NO_3^- and SRP concentrations in the ponds (circles) and in the creek (diamonds).

4.4.5 Biogeochemical processes

Under low-light conditions, NH_4^+ uptake in winter was relatively low (range 0.06 – 0.68 µg N L^{-1} h^{-1}), but consistently measurable (Fig. 4-11 & Table 4-2). Ammonium uptake rates were higher during the melt and the open water periods compared to winter, while the melt and open water uptake rates periods did not differ (Light NH_4^+ uptake: linear mixed effects model; P < 0.001; 45 DF; multiple comparison of means: Tukey test winter:open water – P <0.001; winter: melt – P <0.001; melt:open water – P = 0.12; Fig. 4-11). Ammonium uptake was higher than NO_3^- uptake (wilcox_test; P < 0.001; 106 DF; Fig. 4-12). However, light and dark uptake rates were similar (NH_4^+: wilcox_test; P = 0.87; 70 DF; NO_3^-: wilcox_test; P = 0.15; 34 DF; Fig. 4-

12). Nitrate uptake was not assessed in winter due to low concentrations *in situ*, however, a comparison of NO_3^- uptake rates during the melt and the open water periods showed the melt period had higher rates of uptake (wilcox_test; P = 0.008; 22 DF).

Rates of denitrification were measured in ponds 1 and 5340. The average denitrification rates were very low in winter (2.4×10^{-6} μg N g^{-1} h^{-1}), melt (4.7×10^{-7} μg N g^{-1} h^{-1}) and open water periods (3.9×10^{-7} μg N g^{-1} h^{-1}). Denitrification rates were similar among all seasons at ambient temperature and NO_3^- concentrations (winter 4°C, melt 7°C and open water 20°C; permutation analysis of variance; P ≤ 1, 23 DF). The addition of NO_3^- increased rates in all seasons (wilcox_test; P <0.001; 51 DF), however, there was significant overlap among seasonal rates in NO_3^--amended samples that resulted in only moderately significant differences between amended and non-amended denitrification rates (aovp; P = 0.09, 24 DF). Pelagic nitrification rates were measured in the spring of 2016, when the near-shore environment was oxic. In all three ponds, nitrification rates were at or below their sample specific limit of quantification (average LOQ: 3.3×10^{-3} μg N L^{-1} h^{-1}).

Table 4-2 Mean planktonic nitrogen uptake rates from lakes and ponds in winter. Data are from St. Denis ponds (this study) and subarctic Smith Lake. Nitrate uptake was not measured (NM) in winter in St. Denis. Uptake rates from Smith Lake were obtained from figures in Gu and Alexander 1993. using the 'digitize' function in the R package digitize (Poisot 2011).

Location	Season	Treatment	Ammonium Uptake (μg N h^{-1} L^{-1})	Nitrate Uptake (μg N h^{-1} L^{-1})	Study
St. Denis Pond 90	Winter	Light & 4-hour Incubation	0.155 (0.13-0.17)	NM	This Study
St. Denis Pond 5340	Winter	Light & 4-hour Incubation	0.46 (0.36-0.68)	NM	This Study
St. Denis Pond 90	Melt	Light & 4-hour Incubation	2 (1.4-2.2)	1.18 (1.16-1.3)	This Study
St. Denis Pond 5340	Melt	Light & 4-hour Incubation	49.9 (45.6-50.3)	0.15 (0.12-0.16)	This Study
Smith Lake	Winter	*In situ* light & 4-hour Incubation	1 (<LOQ-25)	<LOQ (NA)	Gu and Alexander 1993
Smith Lake	End of winter/melt	*In situ* light & 4-hour Incubation	22 (3.5-25)	<LOQ (<LOQ-0.7)	Gu and Alexander 1993

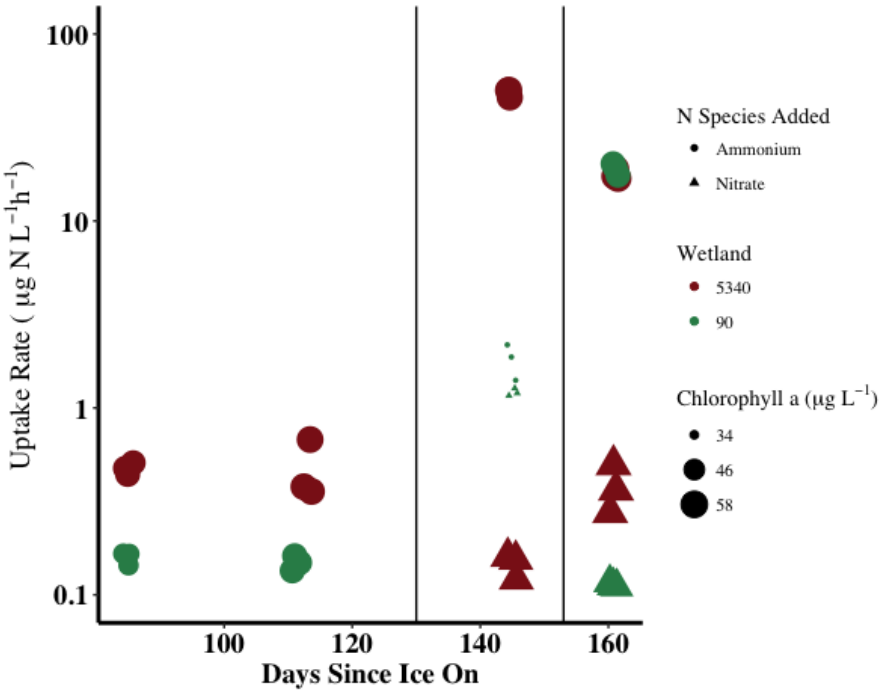

Figure 4-11 Light ammonium (circles) and nitrate (triangles) uptake rates in ponds 90 and 5340 in the winter of 2015/2016. The size of the symbols (circles and triangles) represents the concentration of chlorophyll present at the time of water sampling. Only light and 4-hr incubation presented. The first vertical line represents March 27th, 2016, the onset of spring-melt and the second April 19th, 2016, the day that the ice melted completely from both ponds as defined the by the breakpoint analysis of conductivity.

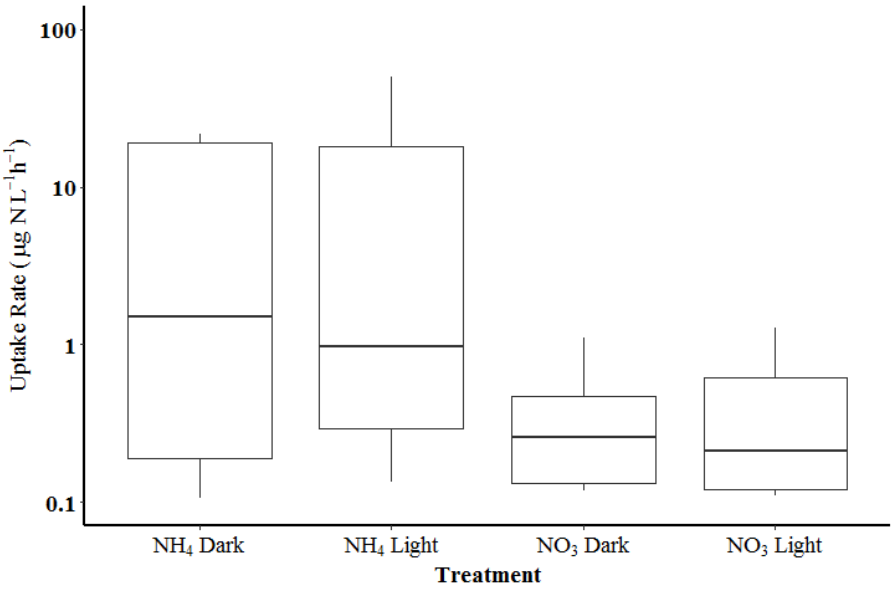

Figure 4-12 Nitrogen uptake rates across the sampling period (winter, spring-melt and open water) for both ponds 90 and 5340 compared between two treatments: NH_4^+ vs NO_3^- (light and dark uptake) and light vs dark (NH_4^+ and NO_3^-). Light conditions were approximately ~30 µmol $m^{-2}s^{-1}$.

4.5 Discussion

Winters are a long, but dynamic period. Rapid biogeochemical change is apparent through winter, with two key periods. First, the wetland ponds are isolated from the surrounding landscape and atmosphere. This isolation leads to rapid oxygen consumption, in most years these ponds experience long periods of low-oxygen conditions, lasting, on average, 3.5 months (range: 2.6-4 months). Ammonium and soluble reactive phosphorus typically increase through the winter isolation phase. With increasing day length and incident irradiance, and warming temperatures, spring-melt begins. This typically induces the influx of oxygen as light stimulates phytoplankton growth, conditions improve for gas exchange, and oxygen rich melt waters enter the ponds. There is greater variability in the influx of oxygen than we would expect (Fig. 4-6). In some years and, in particular, in pond 5340, oxygen concentrations remain low until after the ice is completely gone and, perhaps, wind mixing disrupts salinity induced stratification increasing oxygen penetration into the pond.

This switch to spring-melt is associated with a key period of nutrient decline which occurs despite the nutrient rich inflows. With widespread reports of decreasing duration of ice cover (Mason et al. 2016; Obryk et al. 2016), and high variability in ice cover duration (5-6.3 months; Table 4-1) over just three years in this study, there is growing interest and urgency by scientists to understand the implications of changing ice cover duration to oxygen, nutrients, and aquatic ecology (Agbeti and Smol 1995; Salonen et al. 2009; Powers and Hampton 2016). Here, our data, which encapsulate significant differences in ice cover duration, suggest the phases of winter must be considered in efforts to understand the consequences of declining periods of ice cover. The duration of winter isolation is key to nutrient accumulation, but the switch to oxic conditions, and high light availability during spring-melt may help induce a rapid switch, to low dissolved inorganic nutrients, and higher productivity conditions. This suggests that ice-cover duration and variability may not alter the fate of nutrients, but rather this spring-switch prevents or buffers downstream environments from increased nutrient concentrations.

4.5.1 Oxygen and nutrient dynamics during winter isolation

The observed winter decline in oxygen is chiefly driven by respiration (Bertilsson et al. 2013) while systems are isolated from atmospheric oxygen inputs, inflows and have decreased photosynthetic activity due to low light penetration (Agbeti and Smol 1995; Järvinen and Leppäranta 2011). Shallow lakes, or in this case small ponds, are more susceptible to winter oxygen depletion due to the proportionally greater sediment surface area compared to deeper lakes (Mathias and Barica 1980). The variable duration of oxic conditions in early winter (first 2 months of winter: 2013/2014 – <2 mg L^{-1}; 2014/2015 - 2 mg L^{-1}; 2015/2016 - 7 mg L^{-1}) is likely due in part to differences in autumn conditions. During fall, deposition of organic matter and

warm bottom sediments can interact to increase sediment oxygen demand (Terzhevik et al. 2009). If ice cover forms early, and fall mixing and reaeration are incomplete (due to chemical/salinity caused stratification) (Deshpande et al. 2015), or if there is high organic matter deposition, this could result in earlier and longer periods of winter hypoxia (e.g., 2013/2014). Later ice-cover formation (e.g., 2015/2016 winter), may have positively impacted oxygen concentrations at ice-on and contributed to a shorter period of low-oxygen conditions. Other microbial processes including nitrification may negatively impact oxygen concentrations (Chapter 3; Knowles and Lean 1987; Powers et al. 2017a), although our data suggest this may be a small sink in these systems.

Winter chlorophyll concentrations were generally high in our study ponds (eutrophic to hypereutrophic concentrations according to Wetzel 2001: mean 122 μg L^{-1}, range 2.25-390 μg L^{-1}). However, photo-acclimation to low light conditions leads to high chlorophyll to biomass ratios (Geider et al. 1997; Spilling et al. 2015). Hence, the high chlorophyll concentrations observed under ice are not necessarily comparable to concentrations observed in ice-free periods due to low light acclimation increases in chlorophyll per unit biomass. The implication is that algal biomass per unit chlorophyll is smaller and photosynthetic rates under ice are comparatively limited, especially when the wetlands have snow cover. Light is the key control on phytoplankton growth in these systems and light-limiting snow (Pernica et al. 2017) tends to accumulate in these wetland ponds as they are depressions on the landscape (Pomeroy et al. 1993). Winter algal biomass in these ponds may also be partially controlled by herbivory (Dokulil and Herzig 2009). Larger, potentially herbivorous amphipods (Lindeman and Clark 1999) were present in water samples collected in winter starting in February or March (in field observations, EC), and in spring, migratory bird populations may, in turn, begin to predate the amphipods.

Winter mineralization of organic matter releases nutrients, and redox processes can cause P release from sediment (Catalan 1992; Hampton et al. 2017; Joung et al. 2017). Winter isolation was a period of major changes in nutrient concentrations in these ponds, with an average doubling of NH_4^+ concentrations in surface waters. Indeed, low oxygen concentrations throughout the winter months are associated with this NH_4^+ maxima, likely a result of respiration and mineralization activities (Fig. 4-8a). This is consistent with past work showing increases in winter NH_4^+ (Gu and Alexander 1993) and more recently, higher DIN concentrations observed in shallow lakes in winter (Hampton et al. 2017; Powers et al. 2017b). (Hampton et al. 2017; Powers et al. 2017b). Dissimilatory NO_3^- reduction to NH_4^+ (DNRA) may also contribute to winter NH_4^+ as it occurs under anoxic conditions and contributes to declining NO_3^- (Burgin and Hamilton 2007), as observed during this early winter isolation period – although DNRA process remains unstudied in the prairie pothole region, and would be nitrate-limited through most of the winter period. Although low oxygen concentrations and temperatures may lead to lower mineralization rates (Bridgham et al. 1998; Thamdrup and Fleischer 1998), mineralization is likely the major driver of winter NH_4^+ accumulation (Brooks et al. 1998; Reinhardt et al. 2006), during the winter period of low autotrophic uptake.

Increased NH_4^+concentrations were sustained through winter likely by a combination of low biotic uptake and mineralization release of NH_4^+. Measured planktonic NH_4^+ uptake was low, but it may have decreased the magnitude of NH_4^+ increases observed in winter. Assuming, conservatively, the dark uptake rate for 126 days (average length of the three winters), we estimate that approximately 562 $\mu g\ L^{-1}$ of NH_4^+ was taken up in an average winter (note this does not account for re-mineralization). Compared to the average observed increase in NH_4^+ over winter of 442 $\mu g\ N\ L^{-1}$, this suggests that the net accumulation of NH_4^+ in the water column

underestimates gross mineralization rates, and suggests that planktonic NH_4^+ uptake helps control the magnitude of NH_4^+ uptake through the winter isolation period, although the rapidity of NH_4^+ recycling is not known.

Phosphorus (as SRP) often shows increases in concentration through winter, similar to NH_4^+ for both ponds 90 and 5340 (Fig. 4-8d). The mass ratio of their average increases is low: ~2.1 (from early winter to late winter NH_4^+ increased 464 μg N L^{-1} & SRP increased 209 μg P L^{-1}). While mineralization of organic matter is one phosphorus source, the relatively high phosphorus increase to that of nitrogen suggests anaerobic release of SRP from metal oxyhydroxides in sediments is also important (Nürnberg 1984; Boström et al. 1988; Bridgham et al. 1998). Dissolved inorganic N:P ratios are variable, as noted in Lake Erie particulate stoichiometry ranges from as low as 3.7 to as high as 123; (Prater et al. 2017). This does not reflect the mineralization stoichiometry. Mineralization stoichiometry in soils shows that low ratios of N:P could be associated with P release that exceeds mineralization and is influenced by other geochemical processes (Frank 2008). High anoxic P fluxes occurs in this study system (Armstrong 2018), and suggests that P release from metal oxyhydroxides is an important source of P to the water column. The removal of P from the water column via planktonic uptake, followed by particulate sedimentation can be an important winter process in some lakes (Catalan 1992), although high SRP concentrations combined with low light in winter suggest that it may have only a small influence on observed concentrations.

During winter, NO_3^- was found to frequently decline. This result is in contrast to findings in deeper lakes which retain oxygen through winter and tend to accumulate NO_3^- (Chapters 2 &3; Knowles and Lean 1987; Powers et al. 2017b). Low oxygen conditions in our study ponds inhibit nitrification (Chapter 3; Knowles and Lean 1987). Similar winter declines in NO_3^- have been

observed in a nutrient rich subarctic lake (Gu and Alexander 1993). Causes of observed declines include NO_3^- removal through denitrification, which we know is active during winter (Cavaliere and Baulch 2018), NO_3^- reduction to NH_4^+ (Scott et al. 2008), or possibly abiotic NO_3^- reduction by ferrous iron in the absence of oxygen (Burgin and Hamilton 2007). Autotrophic uptake of NO_3^- may also contribute. Although low light and low NO_3^- concentrations may limit planktonic NO_3^- uptake, the long duration of winter suggests this process does require consideration.

4.5.2 Winter isolation ends with a switch to spring-melt, and rapid biogeochemical change

The month of March is a period of rapid biogeochemical change in these ecosystems – associated with the breakpoint in conductivity and changes in isotopic signature of water (Figs. 4-2 & 4-4). Changes in conductivity were used to identify the earliest period when ice-cover begins to change – with peak conductivity identifying the point at which melt water from the thinking of the ice cover decreases ion concentrations, typically occurring sometime in March (Table 4-1). Melt water from the snow and ice cover on the ponds likely representing the first inputs, followed by stream flows (identified by the timing of changes in the creek isotopic signature; Figs. 4-3 and 4-4). Loss of snow and ice on the ponds is also associated with concurrent increases in light penetration (Salmi et al. 2014). The change in water isotopes comes later, likely driven by inputs from the catchment, with the later timing reflecting a later onset of melt from the catchment, or delayed mixing of the catchment waters below the pond surface.

With melting of snow, and thinning of ice, the spring-melt period represents the end of winter isolation. Ice remains in areas of the pond (Fig. 4-7), but this period is rich in oxygen from atmospheric gas exchange, incoming water and higher rates of photosynthesis. Interannual

variation in oxygen maxima is high. In some of these wetland ponds, it is worth noting, that during this period, a spring oxygen maxima is reached under ice cover due to the ice cover prevention of degassing (Fig. 4-6a,d), while in other ponds, oxygen does not begin to increase until after the ice is completely gone (Fig. 4-6b,c,f). Oxygen supersaturation (>30 mg L^{-1}; within instrument range; Fig. 4-6) is observed in pond 90 in 2015. These concentrations are extremely high, but what we know from other prairie potholes, oxygen concentrations can be high under ice, including oxygen concentrations exceeding 30 mg L^{-1} (lakes in SW Manitoba; Barica and Mathias 1979; Robarts et al. 2005). The differences here are likely due to the different mixing structures that prevail in that particular system; pond 5340 exhibits tell-tale signs of a salinity gradient that likely persists late in the spring-melt period limiting mixing of oxygen-rich waters, while pond 90 begins mixing rapidly, bringing oxygen in, without such a gradient.

Following the NH_4^+ maxima during winter isolation, NH_4^+ declined during the spring-melt and open-water periods. This change is driven by increased NH_4^+ uptake, which is 50 times higher during the melt period than the average uptake during winter. In winter, NH_4^+ uptake, for a given day, could amount to 2.5% of the average *in situ* NH_4^+ concentration. During spring, that daily uptake, for a given day, could amount to nearly 300% of the *in situ* concentration of NH_4^+, suggesting rapid recycling may be occurring for these high uptake rates to be sustained. This is consistent with an increase in autotrophic demand with the influx of light (Table 4-2; Gu and Alexander 1993), and the well characterized spring bloom. Although this spring-melt period is understudied, similarly high uptake rates have been measured during the spring-melt period in a subarctic lake (maxima this study: 50 μg $L^{-1}h^{-1}$; June ice-out maxima in Smith lake: 42 μg $L^{-1}h^{-1}$; Gu and Alexander 1993; Fig. 4-11 & Table 4-2), suggesting it may be common for the spring bloom to occur under ice, and induce major changes in nutrient chemistry. The contrast across

seasons in phytoplankton NH_4^+ demand is clear. While inputs from the catchment resume during the spring-melt period, these inputs had lower NH_4^+ concentrations than the concentrations in the ponds also contributing to decreased concentrations in the pond (Fig. 4-10). Combined, the higher uptake rates (Table 4-2) and lower NH_4^+ inputs from the melt water (Fig. 4-10) explain the observed decline during melt in NH_4^+ concentrations. It is important to note that we measured uptake rates under constant light conditions through the winter, spring melt, and ice-free periods (~30 μmol $m^{-2}s^{-1}$), suggesting that the magnitude of change in uptake rates may be greater than reported here, as light intensities grew during the spring melt, and open water period.

Denitrification is also active during spring, and particularly with the addition of NO_3^- in the melt waters, this could be an important period of denitrification. Snowmelt can be the dominant period of NO_3^- export, with high export associated with soil nitrification under the snow pack (Brooks et al. 1998) and flushing of NO_3^- pools from soils (Seitzinger et al. 2006; Costa et al. 2017). However, the high concentration of NO_3^- in the creek transported melt water is diluted by the large pond volume, limiting the concentrations available to sediment microbes, which are consistently NO_3^--limited (Cavaliere and Baulch 2018). Due to low (unmeasurable) pelagic nitrification during melt, NO_3^- sources may be constrained to those outside inputs, despite the availability of NH_4^+ and oxygen.

Nitrate uptake during melt exceeded NO_3^- uptake during the open water period which is in contrast to previous work demonstrating NO_3^- uptake stays rather consistent in the Alaskan lake as it transitions to the open-water period (Table 4-2; Gu and Alexander 1993). The maximum daily NO_3^- uptake rate, during melt, could consume up to 56% of the average *in situ* NO_3^- concentrations during the same period – hence is a significant NO_3^- sink. While, NO_3^- uptake was higher during the melt period than during the open-water period, it was still lower than NH_4^+

uptake – indicating that preferential use of NH_4^+ is considerable despite the increased likelihood that the autotrophic community has a higher proportion of NO_3^--loving diatoms during the melt period (i.e., spring diatom bloom; Dokulil and Herzig 2009; Glibert et al. 2016).

The observed accumulation of SRP in winter is followed by a decline in SRP during the melt period. This decline is potentially driven by multiple factors including increased oxygen concentrations enhancing the binding capacity of metal oxyhydroxides for phosphate (Orihel et al. 2017) and increased biological SRP uptake associated with increased light (Powell et al. 2008). The melt water, despite the higher SRP concentrations (Fig. 4-10c) does not appear to impact SRP concentrations in the ponds during this period. These findings of a decline in SRP during melt are important, as it appears that biogeochemical mechanisms in the ponds may act over a short time period during spring melt to reduce the concentration of inorganic phosphorus. During this period where connectivity to downstream ecosystems may be at their maxima, biogeochemical processes are driving a rapid decline in this SRP pool, which is assumed to have high bioavailability. However additional work to characterize the fate of this SRP in sediments and water column biota is key to more fully understanding implications to downstream ecosystems.

Dissolved nutrient drawdown is likely an important component of the spring-melt period. As St. Denis Creek flows into the SDNWA, it brings water and solutes into the system. These inputs, we might expect, could alter *in situ* pond concentrations of nutrients through dilution or enrichment. In 2015 over the melt period, Pond 90 increased in depth by 0.5 m according to the pressure level logger deployed in winter of that same year, which is approximately 16% increase in volume. Despite this large volume, we would expect NH_4^+ concentrations to decline by 13%, instead of the steep decline we observed (Figs. 4-8 & 4-10). Similarly, higher

concentrations of SRP and NO_3^- were observed in St. Denis Creek and would amount to 125% and 56% increases of in situ pond concentrations, respectively. However, neither nutrient increases as expected (Figs. 4-8 & 4-10). This indicates, as the uptake results would imply (Fig. 4-11), that there is likely a drawdown effect causing the observed declines in N and P concentrations. If this drawdown in nutrients were to occur regardless of the timing of ice-off, this spring-melt period is likely an important buffer to downstream environments if those dissolved nutrients are effectively transformed and retained within the ponds.

4.5.3 Nitrous oxide dynamics through winter isolation and spring-melt

Nitrous oxide is produced by nitrifying and denitrifying bacteria (Firestone and Davidson 1989). In these ponds, N_2O is typically supersaturated in winter and melt, suggesting one or both of these processes are active. Given our measurements suggest low, or unmeasurable rates of nitrification and denitrification, we highlight the potential importance of a 'leaky' pipe – and high N_2O yields relative to NO_3^- (in nitrification) and N_2 (in denitrification),which can occur under some environmental conditions. Ultimately, N_2O production is controlled both by rates of nitrification and denitrification, and the N_2O yields of these processes. Sulfide inhibition, anoxia and competitive inhibition by methane likely limit nitrification rates (Roy and Knowles 1994; Joye and Hollibaugh 1995). Unpublished data (collected with the N_2O samples) shows that, on average, methane saturation, in winter, is 600%. Although sulfide was not measured, it was likely present due to the high concentrations of sulfate (winter average: 1500 mg S L^{-1}) and low oxygen conditions. While denitrification rates were low during all seasons, incomplete denitrification under low temperature conditions could enhance production of denitrification by-products,

including N_2O (Knowles 1982; Cavaliere and Baulch 2018). Sulfide too can lead to increases in the N_2O:N_2 ratio of denitrification (Sorensen et al. 1980).

Interestingly, the observed supersaturation in winter and spring melt is in contrast to the open water period when these ponds typically function as N_2O sinks. Nitrous oxide undersaturation in the open water period is driven by denitrification, and scavenging of free N_2O in a strongly NO_3^--limited ecosystem. Low NO_3^- concentrations (<38 μg N L^{-1}) have been associated with N_2O consumption (Baulch et al. 2011). Hence our finding of undersaturation is not surprising given that the median concentration in the surface waters during the open-water period is ~40 μg N L^{-1} (note: this is the detection limit for NO_3^-; concentrations are near or below this value). While additional sampling would be required to constrain annual N_2O budgets for these ponds, these temporal shifts in N_2O are interesting, and further suggest that conditions in the pond may not just impact rates of nitrification and denitrification, but also the N_2O yields of these processes. This also suggests that the duration of the open water 'sink' period may be offset by emissions during spring-melt, and that changes in the duration of the winter, melt, and open water periods could affect the role of potholes as sources or sinks of N_2O in the landscape.

4.6 Conclusion

The winter low-oxygen and spring-melt periods are difficult to study, and yet, are critical to understanding how ice-covered wetland ponds might respond to decreased ice-cover duration. Further, understanding year-to-year variability, nutrient dynamics and risks of anoxia during winter could lend insight to changes in water quality – during winter and year-round. During winter isolation, anoxic mineralization of organic matter releases N and P compounds through winter and at the same time metal oxyhydroxides are likely reduced,

releasing P. Conversely, NO_3^- declines in the absence of oxygen, likely due to denitrification (Cavaliere and Baulch 2018) and other processes. Nitrous oxide accumulation, then release during spring melt should be considered when assessing these water bodies as sources (winter) or sinks (summer). Further, nutrient enrichment during fall, prior to ice-on, could lead to enhancement of nitrous oxide production during winter (Baulch et al. 2011).

The onset of spring-melt conditions brings a rapid shift in conditions, with oxygen, light and melt water entering these ponds. Autotrophic uptake dominates changes in N, with maximum NH_4^+ and NO_3^- uptake rates occurring just prior to ice-off, and actually exceeding measurements in the open water season. The influx of oxygen into previously anoxic ponds contributes to a decline in P observed over the melt period that may be geochemically driven, with autotrophic uptake also contributing to SRP decline. Melt water inputs contribute to spring changes, as they are relatively rich in NO_3^- and SRP, but lower in NH_4^+ from the downstream ponds. However, upon entering the wetlands, these nutrients are diluted in the large volume of the permanent wetland ponds. Due to the local hydrology, spring-melt is typically the only time of year when flow can transport nutrients out of many pothole ponds (Cade-Menun et al. 2013; Hayashi et al. 2016). With, the rapidly fluctuating physical and chemical conditions during the melt period, there are major changes in nutrients during this critical moment of water movement. Our findings suggest that the switch to spring conditions, which should precede connectivity, will induce a decline in dissolved inorganic nutrient concentrations from those observed in winter, naturally protecting downstream ecosystems from high export, although it is acknowledged that the transport of other nutrient forms (particulate, dissolve organic matter) may not change meaningfully. As we discuss declining periods of ice cover, and implications both for landscape hydrochemistry and downstream lentic ecosystems, we argue that we must understand the

mechanisms that drive changing conditions through winter, and the individual phases of winter. With this we will better understand how changing winter conditions will affect lake, pond, and reservoir ecosystems, and how the export of nutrients may be altered.

4.7 Acknowledgements

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4.8 Author contributions

E.C. and H.B. developed the study design. E.C. performed the field work, the laboratory analysis, data analysis and wrote the manuscript. H.B. advised at all points during the work and

provided edits on the manuscript. G.K. measured the stable water isotopes and advised on those methods and analysis and interpretation of the water isotope signatures.

4.9 Transition statement

This chapter provides insights to local and short-term nutrient dynamics through winter, the melt period and into the open water season. It combines the analysis of trends in chemistry with process-based measurements within three prairie pothole ponds – marrying some of the measurement techniques used in chapter 2 and 3 to understand more detailed change within several ponds. Chapter 4 is the final chapter that includes my own field-based measurements and lab experiments within this dissertation. In the next chapter (Chapter 5), I took a different approach to understanding winter. I used a long-term dataset to understand changes during winter within a shallow lake, revisiting the idea that there are distinct phases in winter and assessing associated biotic and chemical changes.

5.0 WINTER MATTERS: EVIDENCE FROM A SHALLOW, SEASONALLY ICE-COVERED RESERVOIR

Status: In preparation

Citation: Emily Cavaliere and Helen Baulch. In preparation. Winter Matters: Evidence from a seasonally ice-covered reservoir

5.1Abstract

Winter as a whole has been acknowledged as a key, understudied period in lakes and reservoirs, and a period of rapid change. Using a long-term dataset from a seasonally ice-covered reservoir, we used generalized additive models to assess changes in conductivity, dissolved oxygen, nutrients, phytoplankton and odour during winter and over four decades. Modelling efforts reveal that within this shallow reservoir there were two key phases of winter. Early to mid-winter conditions are characterized by decreasing oxygen, and increasing conductivity, ammonium (NH_4^+) and soluble reactive phosphorus (SRP). Late winter, the month before the ice melts, is a time of fundamental change. Oxygen begins to increase, corresponding to increases in chlorophyll and diatoms. This drives the nutrient drawdown observed at this time. These changes are non-linear. This work informs our understanding that winter is not a homogenous period. Instead, key phases of winter must be considered as we work to understand how diverse lakes respond to winter and will respond to declining periods of ice cover.

5.2 Introduction

The winter period is one of major physical changes. Lakes typically become isolated from atmospheric inputs. Physical processes in lakes differ under ice cover, and of course, water temperatures are cold. Ions are excluded as ice forms, increasing ion concentrations in the water below, a change that can alter mixing patterns (Pieters and Lawrence 2009; Dugan et al. 2017). The availability of light changes through winter, but is typically considered to be low for the majority of winter (Bertilsson et al. 2013); however, sufficient light for under-ice blooms has been observed (Catalan 1992; Twiss et al. 2012; Kim et al. 2015). Water temperatures typically range from 0 to 4°C. Warmer temperatures are generally found near the sediment and cooler temperatures near the ice. Under ice convective mixing can occur when light penetration is sufficient or when heat flow from the sediments induces currents (Pernica et al. 2017). Similarly, water moving into the lakes under ice cover can cause mixing (Bengtsson 1996), although often the volume of surface inflows typically decreases in winter. These physical changes are linked to important biological changes. Photosynthetic production is often limited by light penetration which in turn is largely limited by snow cover (Bertilsson et al. 2013), although periods of low snow cover can increase phytoplankton growth (Catalan 1992; Pernica et al. 2017). Where photosynthetic oxygen production is low, the winter isolation of a lake from the atmosphere can lead to accumulation of respiratory gases, and loss of oxygen (Chapter 4; Fang and Stefan 1997; Denfeld et al. 2018).

Nutrient concentrations also change in winter. Nutrients may be relatively plentiful (Chapters 2-4; Hampton et al. 2016), due to low nutrient uptake and continued mineralization supporting higher concentrations of NH_4^+ (Chapter 4; Catalan 1992), which in turn can generate NO_3^-

through nitrification (Chapter 3; Powers et al. 2017b; a). While nitrification may generate NO_3^- from NH_4^+ if sufficient oxygen is available (Chapter 3; Powers et al. 2017a), there is evidence that denitrification could permanently remove NO_3^- from water bodies even in winter (Grantz et al. 2012; Myrstener et al. 2016; Cavaliere and Baulch 2018). Further, winter low oxygen conditions could contribute to increases in available phosphorus (Orihel et al. 2017). Previous winter work has often focused on short-term (a year or a few year study) observations or differences between seasons (Knowles and Lean 1987; Catalan 1992; Myrstener et al. 2016; Hampton et al. 2017). In addition, much of what we know is from relatively deep lakes, and lakes with long residence times, begging questions about whether shallow lakes may be more dynamic in winter. Here we use a long-term water quality data set from a shallow reservoir in Saskatchewan that is used as a source to supply drinking water. We assess how physical, biological and temporal changes affect water quality during winter.

5.3 Methods

5.3.1 Study site

Prairie lakes, ponds and reservoirs are often shallow, and are characterized by high nutrients, as is the case for Buffalo Pound Lake, which is the source of drinking water for the cites of Moose Jaw and Regina (Buffalo Pound Water Administration Board 2010). Buffalo Pound is fed by the Qu'Appelle River which flows east and south from Lake Diefenbaker (dammed reservoir approximately 100 km upstream) (Parsons et al. 2012; Swarbrick et al. 2018). It was formed by the damming of a long shallow lake, which now has a mean depth of 3 m (Hall et al. 1999). Buffalo Pound Lake is surrounded by agricultural fields (75% of the drainage basin). The residence time of this mesotrophic, polymictic lake (Vogt et al. 2018) varies widely depending on

hydroclimate and management, but the average residence time is ~8 months. The lake is subject to long ice-covered periods and is in a semi-arid region with a snowmelt-dominated runoff regime (Robarts et al. 2005; Nachshon et al. 2013) where cyclic wet-dry periods are associated with flood risk and with severe drought (Wheater and Gober 2013).

The major water source of Buffalo Pound Lake is from inter-basin water transfer from Lake Diefenbaker with smaller inputs from the local Qu'Appelle catchment, principally during snowmelt. However, in some years the local catchment contributes a substantial proportion of the annual flows to Buffalo Pound. Water levels in the lake are tightly controlled (Hall et al. 1999). In addition to its role as a crucial regional drinking water supply, it is a site of intensive recreational activity, including a provincial park, cottagers, active recreational fishery in summer and winter months and an industrial water source. The long-term data set used here was analyzed and collated by the Buffalo Pound Water Treatment Plant (BPWTP) since the 1970s. The data for this study begins in 1977 and ends in 2016. It includes different biological and chemical data from intake raw water samples (Table 5-1). Dates of ice formation and ice melt were also recorded. Some variation in the mix of water sources occurs seasonally, with two inlets comprising the water supply (located approximately 170 m apart, at 3 and 2 meters below the surface of the reservoir).

5.3.2 Treatment plant methods

Water samples were collected from two intake pipes around 1-m above sediments. The raw water samples were analyzed the same day of collection and analyzed according to the following methods (Table 5-1; APHA 2012). Specific conductance was measured, with internal temperature corrections, on a conductivity meter. Dissolved oxygen was measured in lab with an oxygen

probe. Ammonium was analyzed using the colorimetric 4500-N-NH3 A2 method as in Standard Methods for the Examination of Water and Wastewater (APHA 2012). Sulfate and NO_3^- were analyzed via ion chromatograph modified from the SM 4110B method (APHA 2012). Soluble reactive phosphorus (SRP) was analyzed using the NH_4^+ molybdate method and analyzed on a spectrophotometer (APHA 2012). Chlorophyll was extracted with acetone and analyzed using a spectrophotometer (APHA 2012). Chlorophyll data were not corrected for the presence of pheophytin. The threshold odour number was determined based on APHA (2012; 2150 method) via sequential dilutions of raw water. Dissolved organic carbon was determined by acidification to remove inorganic carbon. Then the sample is injected with sodium persulfate and treated with ultraviolet light which transforms the organic carbon to CO_2 which is then analyzed on a non-dispersive infrared detector (APHA 2012). Algal counts (diatom, green algae and cyanobacteria) were performed on raw water by trained staff, but not taxonomists, with taxa identified by comparison with accepted descriptions of organisms. The frequency of sampling has varied through the time series, and different analytes were added to the routine monitoring at different times (Table 5-1). For example, consistent SRP analysis did not begin until the 1980s while chlorophyll analysis did not begin until the mid-1980s. In the early 2000s, NO_3^-, NH_4^+ and oxygen were sampled at decreased frequencies (from every week to every other week). Taxonomic counts began in 1995.

Table 5-1 Analytical methods (APHA 2012) used at the BPWTP, periods of measurement and number of data points (N) in the total database and number of data points (N) in the ice-covered period. The accredited BPWTP laboratory did change instruments over the long history of this dataset.

Analyte	Method	Period of measurement	N total	N ice-cover period
Conductivity	Conductivity Meter; Methods Manual #6, v. 2.14	1978-2015	1455	564
Dissolved Oxygen	Oxygen Probe; Methods Manual #22, v. 1.2	1977-2015	1699	647
Chlorophyll	Acetone Extraction and analysis on spectrophotometer; Methods Manual # 206, v. 2.1	1979-2015	1177	381
Nitrate (NO_3^--N)	SM 4110B – Method Manual #3, v. 2.3	1985-2016	768	329
Ammonium (NH_4^+-N)	Colorimeteric 4500-NH_3-N A2 – Methods Manual #201, v. 2.2	1977-2016	1308	445
Soluble Reactive Phosphorus (SRP)	Ammonium Molybdate – Methods Manual #203, v. 3.1	1978-2016	1157	431
Diatom, Green Algae and Blue-Green Algae (cyanobacteria) Count	Raw water counts – Methods Manual #204, v. 2.1	1995-2016	996	306
Odour	Threshold odour number – Methods Manual # 2150	1978-2014	1750	652
Dissolved Organic Carbon	Tekmar/Dohrmann Phoenix 8000 Carbon Analyzer – Methods Manual # 20, v. 2.1	1986-1990; 1997-2004	582	218

5.3.3 Statistical methods

All statistical analyses were performed using R: A Language and Environment for Statistical Computing, version 3.5.0 (2018-04-23; R Core Team 2018). Prior to analysis, the variables of interest were normalized to the mean for that variable for that specific year. All models and model plots, therefore, use the normalized values as they relate to the specific annual mean of that data point; values greater than one are higher than the annual mean and values less than one are lower than the annual mean. Diatom counts were modeled using the raw count data and not normalized to the annual mean. Only the period of winter is modeled in the following analysis; the period of winter is defined as the ice formation and ice break up date as recorded by the BPWTP (near the intake). Generalized additive modelling (GAM) and general linear modelling (GLM) were used to identify which water quality parameters were the best covariates for

particular response variables in winter. These two model approaches allow the response variable to have response error distribution other than normal distribution, including gamma, Poisson, log, etc. (Wood 2017). These models work in a similar way to simple straight-line linear model by establishing a relationship between the response variable and predictor variables, but with added flexibility not limited to normal distribution of errors (Wood 2017). The GAM, instead of fitting a linear relationship like in GLM, fit a linear predictor that depends on a user-selected smooth function of predictor variables. The linear predictor is the best estimate of the response variable as it fits in the GAM. Gamma, inverse or log distributions were used because our data are biased towards the lower numbers. For example, conductivity is normally distributed in winter based on a frequency histogram, so the model uses the inverse (or normal) distribution. To model the diatom count data, we used the more traditional GAM mgcv package with the Poisson distribution and Tweedie link ('gam' function; mgcv package; Wood 2011). The diatom data had a Poisson distribution (discrete distribution) rather than a continuous distribution as used for the other variables of interest. The distribution family and link for the GLM were similarly determined depending on the response variable distribution ('glm' function; stats package; R Core Team 2018). Table 5-2 shows the model components of interest from the GAM and GLM models.

The GAM models use this common notation to signify model structure:

$$y_i = \beta_o + f_1(x_{ai}) + f_2(x_{bi}) + \ldots + f_n(x_{ni}) + \text{residuals} \quad (5.1)$$

Where y_i is the response variable, β_o is the intercept and $f_1(x_{ai})$ and $f_2(x_{bi})$ are smooth functions for explanatory variables a and b. Each of the explanatory variables explain some proportion of the variance of the response variable. The larger the estimated degrees of freedom (EDF), the greater the impact on the response variable (Wood 2017). There is substantial overlap between

the covariates identified as important in the two model types. The GAM results are graphically presented in the results because the GAMs are capable of assessing non-linear relationships, which appear to be of particular importance in winter.

The GLM were optimized using the AIC (Akaike information criterion) to find the best model (lowest AIC). The GAM were optimized by comparing model REML (restricted maximum likelihood) and selecting the lowest REML (Wood 2017). This often meant that while some explanatory variables may not be significant ($P > 0.05$), they were included in the final model selected to minimize AIC or REML and maximize variance explained. In addition, the model residuals and fitted values were assessed for normality and excluded if visual inspection failed the test of normality. The GAM were also built to minimize concurvity, which is a measure of the co-variance of the explanatory variables, similar to co-linearity in linear models ('concurvity' function; mgcv package; Wood 2004). Temperature was removed from several models due to the strength of the relationship between days since ice on and temperature. The type of GAM model was censored for nutrient and chemical variables (‘gam’ function; cen.GAM package, Fang 2017). To reiterate, the GAM presented here are the best models that minimized REML, maximized variance explained, and had the fewest variables (with little or no concurvity present) with the most normal residuals. GLM results are also reported (Table 5-2) and reflect similar covariates of interest for the response variables.

The models presented here exclude previous, more heavily parameterized versions. For example, the odour model originally had green algae, blue-green algae (cyanobacteria) and diatoms as model parameters. These were included due to previous work with this dataset indicating that these species would be important to odour (Kehoe et al. 2015). However, in order to simplify this model, these parameters were removed and instead, higher impact parameters

were only retained (high EDF – SRP, DIN and year) associated with the lowest AIC/REML model (Table 5-2). Other models were eventually excluded, including the model for green algae which was excluded due to poor fit and low adjusted R^2 (0.07) and organic carbon which was excluded due to limited available data (Table 5-1). Diatoms were the only group where informative models were produced, hence results for cyanobacteria and green algae were excluded from the reported model outputs.

5.4 Results

The date of ice-formation and complete ice-off varied widely from year-to year leading to a more than 50d range in the duration of ice cover over the 39-year period of the data record (Fig. 5-1). The raw data reveal some qualitative trends (Fig. 5-1). Water temperatures in winter (under ice) ranged from 0.5-9.9°C and tended to increase at the end of the winter period (Fig. 5-2). Water quality shows important seasonal changes in some parameters. The lowest oxygen values were commonly observed in winter, but summer low oxygen conditions are also common in this lake. There was high winter variability in oxygen concentrations, but a tendency for declining oxygen through the early stages of winter. Nitrate minima were commonly observed in spring and summer, and NH_4^+ concentrations tended to be highest in winter (median = 0.12 mg L^{-1}). Two different SRP maxima occur –one in winter and one in summer. Odour peaks in summer, although late winter peaks can also occur (Fig. 5-2). Diatom abundance was uniformly low until late winter when abundance increased (Fig. 5-2 & Appendix B Fig. C-4-1). Cyanobacterial abundance was also low throughout winter, with the exception of a few data points (Fig. 5-2 & Appendix B Fig. C-4-1). Green algae were often

the dominant taxa through winter, often exceeding 80% of the total phytoplankton abundance (Fig. 5-2 & Appendix C Fig. C-4-1).

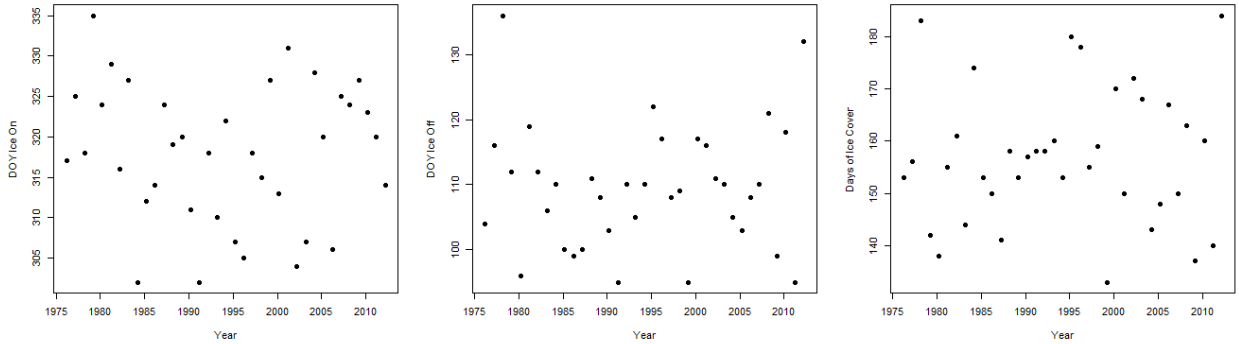

Figure 5-1 Day of year of ice formation and ice melt and the corresponding days of ice cover.

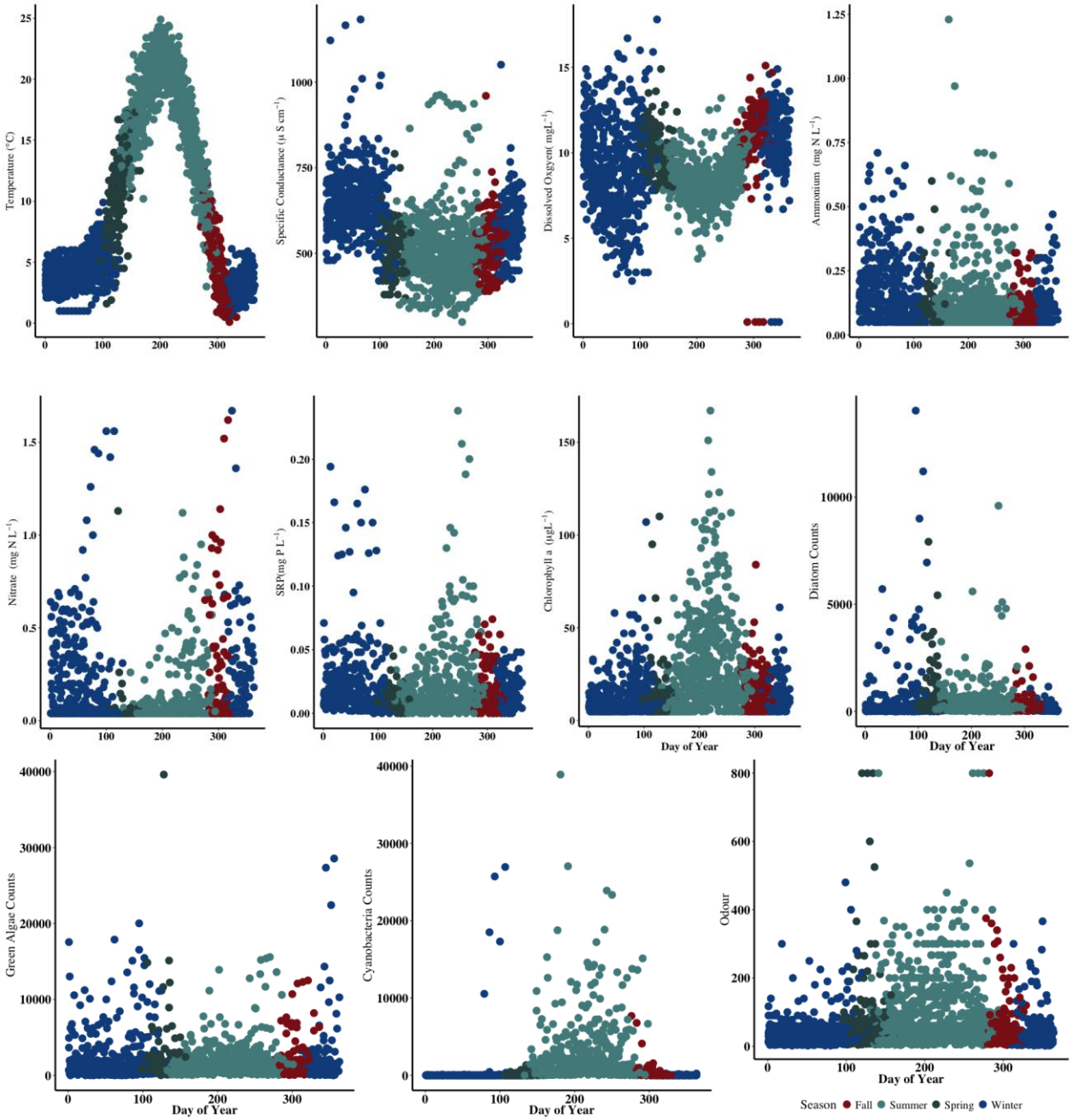

Figure 5-2 Seasonal variation in concentrations of temperature, conductivity, dissolved oxygen, NH_4^+, NO_3^-, SRP, chlorophyll, diatoms green algae, blue-green algae, and in threshold odour number throughout the data record. The seasons are defined as winter: duration of ice cover; spring thaw: month after ice-off; summer: from spring until fall; fall: month before ice-formation. Note typical (median) ice cover duration end on 110 day of year, while ranges from 94 to 136 day of year. Data presented span the entire available data record (described in Table 5-1), of up to 39 years.

Modeling of the normalized and count data (diatoms) allow us to evaluate within winter trends, and which variables appear to be the strongest covariates (Table 5-2; Figs. 5-3, 5-4, 5-5, 5-6). Days since ice on was a strong (largest EDF) and significant covariate in the GAM of conductivity, oxygen, NH_4^+, SRP, chlorophyll, and diatoms (Fig. 5-3), while year also appeared in multiple models (Fig. 5-4, Table 5-2). This inclusion of year is consistent with observations of long-term annual changes in the open water season (Fig. 5-4), for example a cyclic fluctuation in SRP, with evidence of an increase in recent years. Most response variables exhibited a directional change near the end of winter. Using these model fits for each of these response variables, we found what day (as days since ice on) the first maximum or minimum fit value falls upon ('which.max' or 'which.min'; function; R Core Team 2018). This day, or transition point varies among the variables of interest; conductivity peaked reliably, for each year, between 98 and 104 days since ice on, with the average around 101 days since ice on. Oxygen concentrations tend to decline over winter, reaching a minimum, on average around 102 days since ice on. However, this minima can occur at almost any time in winter, between 9 and 160 days since ice on. Ammonium and SRP reached maximum concentrations on average at 70 and 63 days since ice on respectively, again with a wide range of dates for the maxima (both: between 9 and 113 days since ice on). Around the same time that NH_4^+ and SRP peaked (Fig. 5-3), chlorophyll concentrations and diatom counts reached their minimum (average, 69 and 64 days since ice on respectively; minima between 6 and 123 days since ice on).

Oxygen explains some of the variability in models of NO_3^-, NH_4^+, and SRP (Fig. 5-5; Table 5-2). Specifically, NO_3^- is inversely related to oxygen. SRP shows a similar relationship – declining at higher oxygen (oxygen values greater than 0.6 of the annual oxygen mean; Fig. 5-5), although this relationship only becomes apparent at moderate oxygen concentrations. Finally,

NH_4^+ declines at higher oxygen concentrations (oxygen values greater than 0.6 of the annual oxygen mean; Fig. 5-5), although again, the shape of this relationship differs at lower oxygen concentrations (oxygen values less than 0.6 of the annual oxygen mean; Fig. 5-5). Interestingly, an interaction between NH_4^+ and NO_3^- is associated with oxygen concentrations (Table 5-2). Nitrate does not show a relationship to days since ice on. Instead, variability is explained most substantively by year (substantiated by Appendix C Fig. C-4-3) followed by dissolved oxygen. SRP is also impacted by year followed by dissolved oxygen and days since ice on. Days since ice on is the most important variable in predicting oxygen concentrations, followed by the interaction between NO_3^- and NH_4^+ (Table 5-2).

Dissolved inorganic nitrogen (DIN – NO_3^- plus NH_4^+) and SRP appeared in models for algal related parameters (Fig. 5-6; Table 5-2). Higher chlorophyll concentrations are associated with lower DIN and SRP (DIN and SRP less than 1 of the annual mean), but at higher nutrient concentrations, this trend weakens or disappears (DIN and SRP greater than 1 of the annual mean; Fig. 5-6; Table 5-2). Relationships between diatoms and nutrients (DIN and SRP) are more complicated, with somewhat weaker model fit. Odour covaried with both DIN and SRP showing winter maxima at low nutrient concentrations. As noted, models of cyanobacteria and greens had poor explanatory power, and results were not reported for model-based analyses.

Table 5-2 Model covariates with best fit (R^2) and converged, order of term indicates strongest to weakest model contribution or estimate (GAM - EDF or GLM - weight). NA indicates not applicable. Other abbreviations are: SRP (soluble reactive phosphorus), DIN (dissolved inorganic nitrogen), GAM (generalized additive model), and GLM (generalized linear model).

Variable*	Significant covariates in GAM†	Non-Significant but still in model†	GAM Adjusted R^2	Significant covariates in GLM†	Non-Significant but still in model †	GLM Adjusted R^2
Conductivity	Days	NA	0.29	Days, days*year	Year	0.23
Oxygen	Days, nitrate*ammonium, ammonium, nitrate	NA	0.30	ammonium, flow, nitrate, days, year, chlorophyll, temperature	Days, ammonium	0.53
Nitrate	Year, dissolved oxygen	Ammonium, temperature	0.21	Temperature, ammonium*temperature, dissolved oxygen, ammonium	Days	0.27
Ammonium	Days, SRP, dissolved oxygen, organic carbon	NA	0.45	Organic carbon, dissolved oxygen, days	NA	0.93
SRP	Year, dissolved oxygen, days, organic carbon	NA	0.48	Organic carbon, dissolved oxygen, days	NA	0.89
Chlorophyll	Days*year, days, DIN, SRP, year	NA	0.31	SRP, year, days	DIN, DIN*SRP	0.49
Diatoms	Days, DIN, temperature, SRP	NA	0.23	DIN, days	NA	0.53
Odour	Year, DIN, SRP	NA	0.20	SRP, year	DIN	0.70

†Significant covariates were identified based on a significance level of $P<0.05$. Nonsignificant model terms were included if removing the term reduced model fit (AIC, R^2, or residuals)
*Note that cyanobacteria and green models are not reported due to poor model fit.

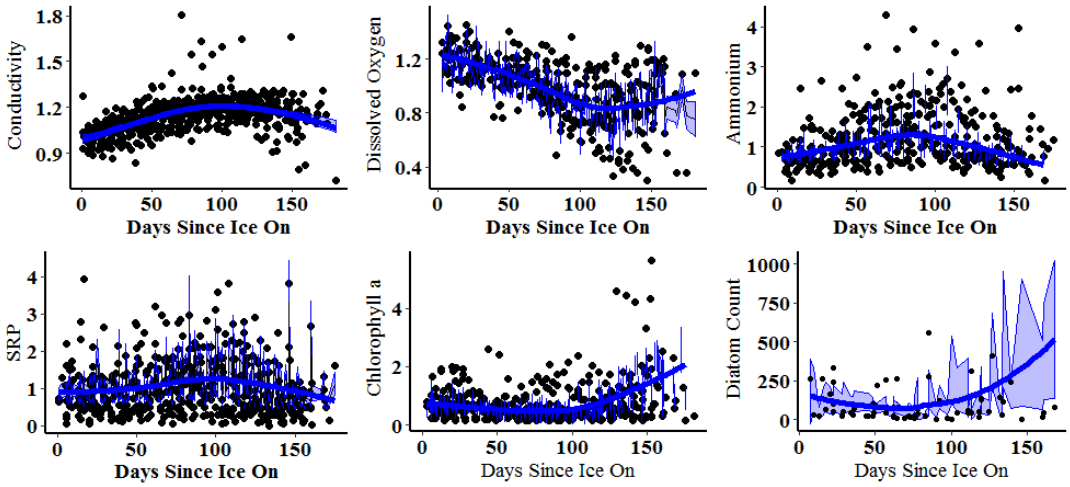

Figure 5-3 Loess fit of GAM predictive model and normalized data for under-ice conductivity, oxygen, NH_4^+, SRP, and phytoplankton (as chlorophyll and diatom counts) as they change during the ice cover period (Days since ice on). Individual points are normalized to the annual mean (conductivity, dissolved oxygen, NH_4^+, chlorophyll) or count (diatoms) response variables as they change over the winter (as days since ice on). The smooth blue lines are loess lines of the GAMs and the blue shaded regions (or wiggly lines) are two standard errors away from the fit line.

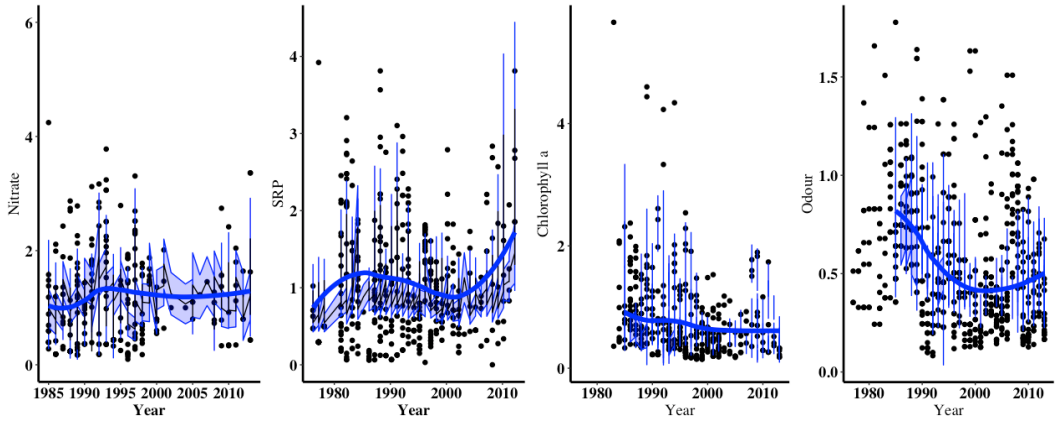

Figure 5-4 Loess fit of GAM predictive model and normalized data for under-ice NO_3^-, SRP, chlorophyll, or odour as they change over the years. The smooth blue lines are loess lines of the fit models and the blue shaded regions (or wiggly lines) are two standard errors away from the fit line.

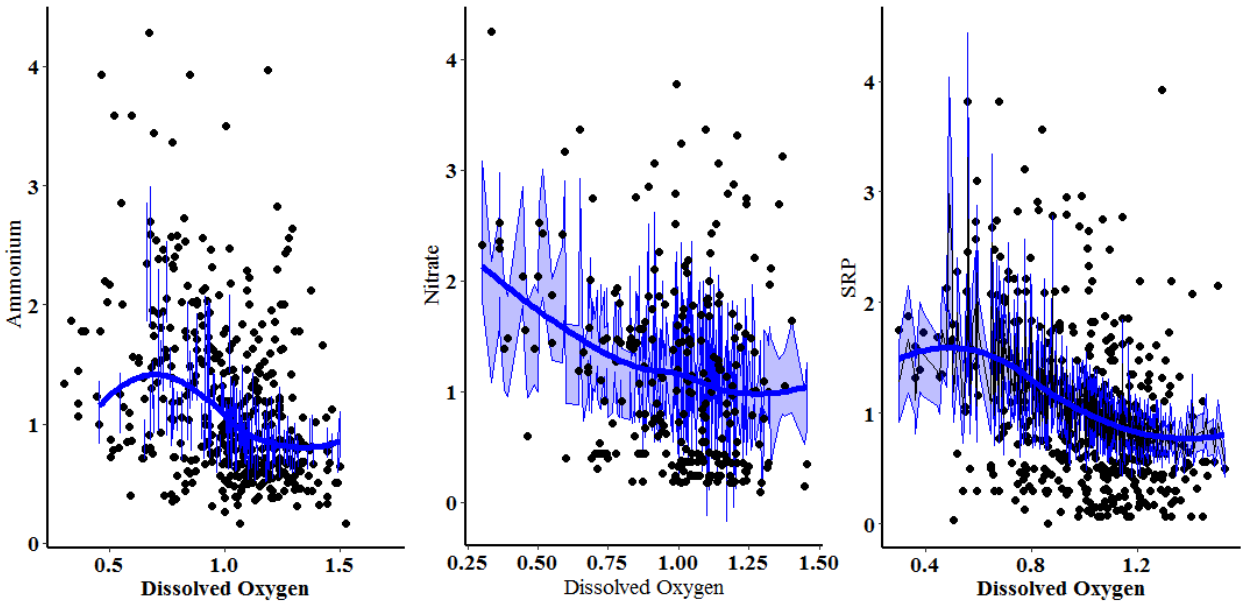

Figure 5-5 Loess fit of GAM predictive model and normalized data for under-ice NH_4^+, NO_3^- or SRP as they change with dissolved oxygen. The smooth blue lines are loess lines of the fit models and the blue shaded regions (or wiggly lines) are two standard errors away from the fit line.

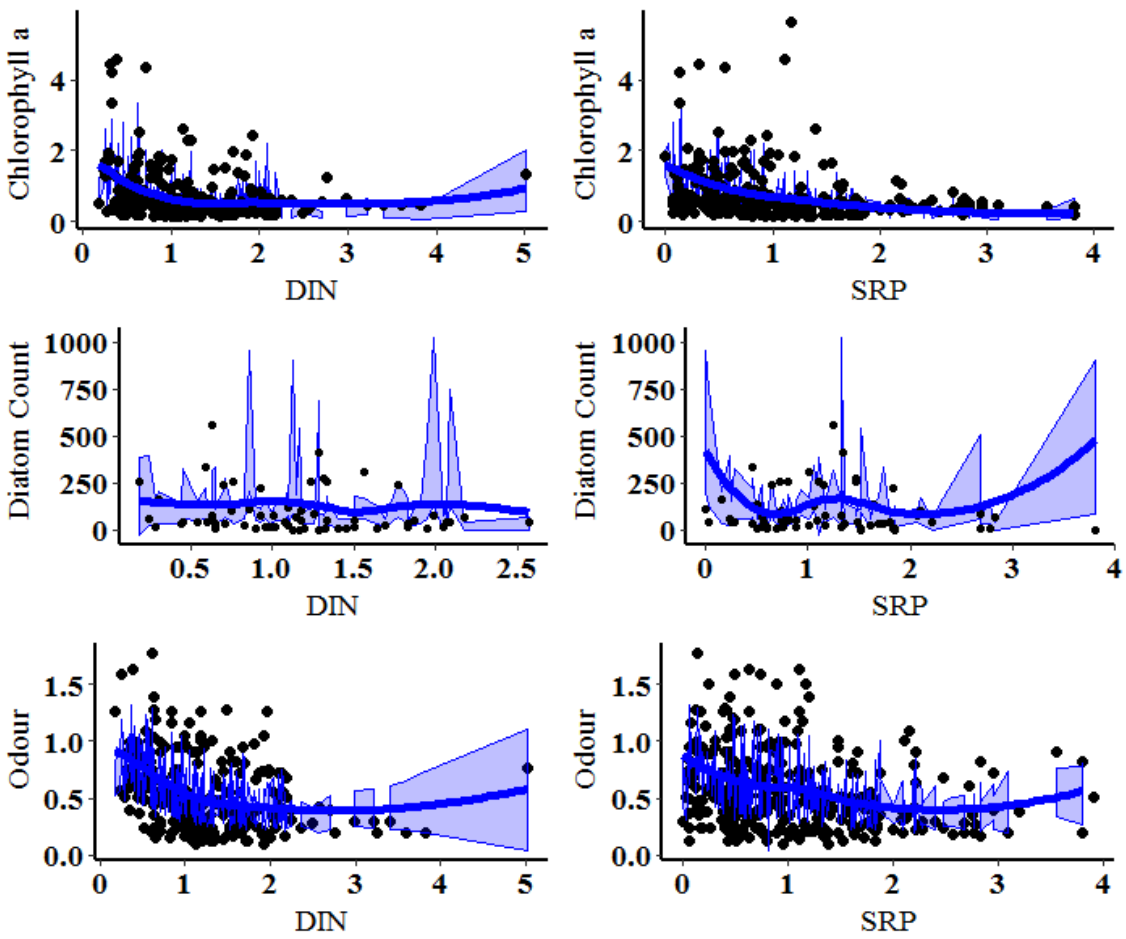

Figure 5-6 Loess fit of GAM predictive model and normalized data for under-ice normalized chlorophyll, diatom counts and odour as they change with DIN or SRP. The smooth blue lines are loess lines of the fit models and the blue shaded regions (or wiggly lines) are two standard errors away from the fit line.

5.5 Discussion

Temporally intensive, long-term, winter data are rare, yet provide unique insights into the understudied winter season, how conditions change through winter, and how lakes may change with shortened ice cover periods. We demonstrate, through modeled trends and observations that important changes in chemical and biotic conditions occur during winter. An important ecosystem service, the treatability of drinking water, can be impacted by winter conditions with major odour events periodically occurring near ice-out, concurrent in rises in phytoplankton numbers. Days since ice on explains many of the changes observed in this reservoir during winter

(large EDF, Table 5-2 & Fig. 5-7), suggesting ice cover duration may be important to lake chemistry and productivity. However, fully understanding the effects of ice cover duration will necessitate understanding intricacies of key periods of change during winter, the drivers of winter changes, and the interactions among chemical, physical, and biological parameters. Models of nutrients, oxygen and biological variables show often strong connections – oxygen relates to nutrient chemistry, and nutrient chemistry relates to biological variables that are linked to productivity (Fig. 5-7). However, here it appears that higher productivity is drawing down nutrient supplies under ice. As expected, modeled oxygen is linked to indicators of productivity – with seasonal shifts in oxygen, chlorophyll and diatoms suggesting an important, late winter increase in productivity. Although peaks in winter chlorophyll are lower than in the summer bloom season, they exceeded 100 $\mu g\ L^{-1}$ in late winter (Fig. 5-2). Chapter 4 further illustrates the capacity for increases in productivity under ice and late winter (or melt period), nitrogen uptake, and the associated drawdown of dissolved nutrients. Work in deep lakes has shown similar late-winter trends of increased under-ice productivity (Katz et al. 2015; Hampton et al. 2017), and it appears that those trends may also be true in shallow lakes, although productivity was not directly measured here.

The temporally intensive data also reveals long-term fluctuations. The hydrological cycle of the prairies is defined by its extreme variability (Fleming and Sauchyn 2013; PaiMazumder et al. 2013), and shallow lakes are typified by their tendency to change quickly (Feuchtmayr et al. 2009; Golosov et al. 2012). Not surprisingly, over the time series we observe shifts in NO_3^-, SRP, chlorophyll and odour, reflected in 'year' appearing as a covariate in several of the models (Fig. 5-4). Nitrate concentrations have changed dramatically over the four decades of monitoring (see Appendix C Fig. C-4-3). SRP concentrations have tended to fluctuate over the time series; higher

concentrations were common in the 1980s (mean: 32.5 mg L^{-1}), while the 1990s had fewer peaks (mean: 18.7 mg L^{-1}) and SRP in the 2000s was commonly lower (mean: 11.5 mg L^{-1}). The wet and dry cycles of prairie hydrology may impact internal and external nutrient loading via changes in water flow paths and water sources, temperature and oxygen status (D'Silva 2017; Orihel et al. 2017; Ryberg 2017), contributing to the large changes observed here in NO_3^-, SRP, and subsequent changes in chlorophyll and odour in recent years (Kehoe et al. 2015, Fig 5-4).

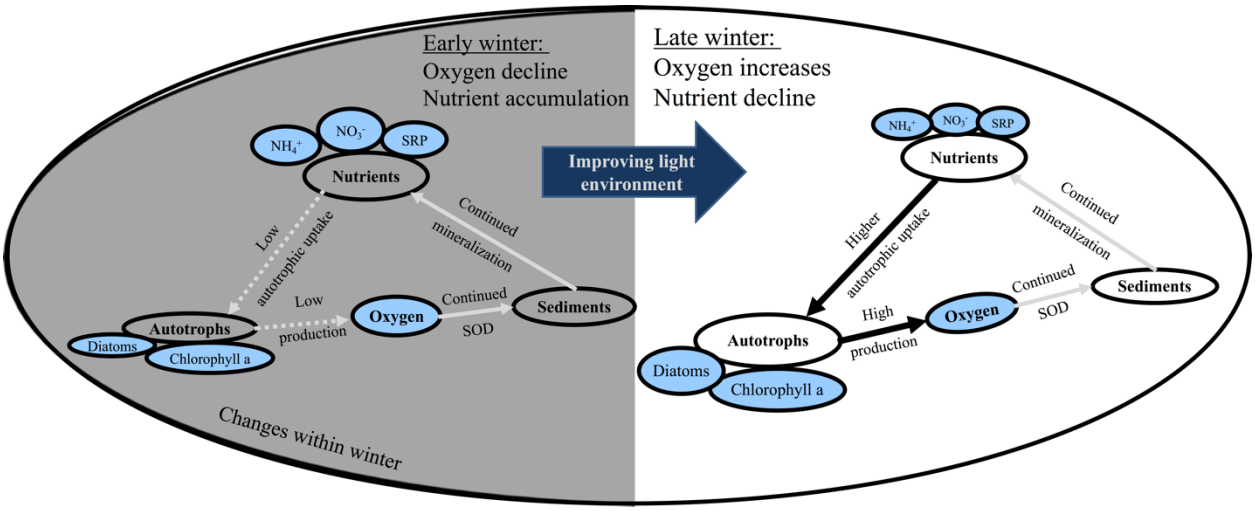

Figure 5-7 Schematic of observed changes from early winter through late winter, and hypothesized drivers of change within Buffalo Pound Lake, a shallow, productive reservoir. The early winter phase is defined by oxygen decline, and nutrient accumulation. Sediment oxygen demand and nutrient mineralization is assumed to be constant through the phases, but low autotrophic demand allows accumulation of nutrients in early winter. Low photosynthetic oxygen production does not balance oxygen demand, leading to oxygen declines. As the light environment improves, the late winter phase is typified by higher oxygen associated with high autotrophic oxygen production (which is not released to the atmosphere due to ice cover), and high autotrophic nutrient demand, drawing nutrient drawdown. Oxygen changes can also cause geochemically driven-changes in nutrients, for example associated with release of metal-bound phosphorus under anoxic conditions, and sorption/precipitation later in winter as oxygen re-enters the system. Measured variables are indicated in light blue, with the size of the oval reflecting the relative size of the pool or community. Arrows are weighted and coloured to reflect the hypothesized relative importance of processes with dashed grey arrows showing very low rates, blue arrows showing intermediate rates, and black arrows showing high rates.

Phases of winter – early winter

Early modelled winter changes, as captured by the covariate days since ice on, were characterized by increases in conductivity, NH_4^+, and SRP, and decreases in dissolved oxygen,

NO_3^-, chlorophyll and diatoms (Fig. 5-3). Specific conductance increases as ice forms through the first phases of winter due to ion exclusion from the growing ice cover (Dugan et al. 2017; Chapter 3), although interestingly this physically-driven process showed relatively poor model fits. This could relate to groundwater inputs changing conductivity in the lake. Oxygen depletion in winter is a well-documented phenomenon, particularly in shallow, nutrient-rich lakes (Barica and Mathias 1979; Mathias and Barica 1980; Robarts et al. 2005) resulting from limited gas exchange, respiration and low autotrophic activity (Mathias and Barica 1980). Declining chlorophyll and declining abundance of diatoms suggest lower autotrophic activity during early winter (non-diatom taxa were not modelled). This is particularly true in light of increasing chlorophyll:biomass ratios often observed in winter ocean environments (Arrigo and Thomas 2004; Hodal and Kristiansen 2008), and lakes (e.g., Catalan 1992) associated with acclimation to low light levels. The extremely low rates of NH_4^+ uptake observed in the early winter nutrient accumulation phase (Appendix C Table C-4-1) suggest that mineralization is the key driver of accumulation. Autotrophic uptake does not appear to constitute a significant sink for dissolved nutrients during the early winter phase.

Oxygen depletion rates are greater in more eutrophic systems and in shallow lakes (greater sediment oxygen demand per unit water volume, Mathias and Barica 1980; Meding and Jackson 2003). In systems with a mean depth of less than 3 m, fish kills are common while if the mean is greater than 5 m, winter oxygen depletion results in fewer fish kills (Barica and Mathias 1979). Buffalo Pound seems to diverge from these general trends. Despite its shallow depth, Buffalo Pound has not experienced recent winter fish kills, although fish kills have occurred historically (Hammer 1983). This low anoxia risk may be due to its long fetch, limited snow accumulation,

combined with the continuous (although low) winter flow, (continuous flow; Kehoe et al., *in prep.*), and small ice-free areas.

Concurrent with early winter declines in oxygen were increases in NH_4^+ and SRP. These early winter increases seem fairly common in the literature across both shallow and deep lakes, and across varied trophic statuses (Catalan 1992; Gu and Alexander 1993; Niemistö and Horppila 2007; Powers et al. 2017b) and were observed in the ponds of Chapter 4 as well. In a large synthesis study of 101 lakes, winter increases in dissolved organic nitrogen concentrations were observed and attributed to mineralization of organic matter (Hampton et al. 2017). Mineralization is likely important here to NH_4+ increases. Moderate increases in SRP or TP have also been attributed to mineralization of P (Catalan 1992). Recent work found that there was low to moderate internal loading of phosphorus in winter in Buffalo Pound (D'Silva 2017). Nitrate concentrations in Buffalo Pound tend to vary widely in winter, however, late winter peaks have been observed in some years. Several other works have observed NO_3^- increases over winter and attributed these changes to winter nitrification (Chapter 3; Powers et al. 2017a; b), while other studies report slight NO_3^- declines or no trends similar to this study (Chapter 4; Catalan 1992; Gu and Alexander 1993). This difference in the dynamics of NO_3^- in winter is likely a result of the spatially and temporally variable nitrification. As found in Chapter 2, nitrification can occur through winter, and relationships between NO_3^-, ammonia, and oxygen are indicative of a potential role for nitrification here, although, the lack of clear winter changes in NO_3^-, as shown in other ecosystems, may relate to interannual and seasonal variability in NO_3^- sinks (including autotrophic uptake), and sources, through the dynamic winter period.

Late Winter Transition

In late winter, the models show that the earlier trends of declining oxygen, chlorophyll and diatom counts begin to reverse themselves. Early winter trends of increasing conductivity, NH_4^+ and SRP likewise begin to reverse direction. Light conditions change over the course of the winter as snow accumulates, and melts or is blown from the lake surface (Catalan 1992; Malm et al. 1997), and we hypothesize changing snow and light conditions are important to the observed changes. Blowing snow, acutely important in the Prairies, can have dramatic effects on light penetration – either blowing snow onto the lake (in a depression – as would happen in the ponds in Chapter 4) and limiting it or by blowing the snow off the lake, particularly in a long lake like Buffalo Pound. In late winter, changes in light associated with longer day length, a higher angle of the sun, and decreasing snow cover, combined with warming water temperatures, likely stimulated productivity increases, driving these late winter transitions.

The late winter transition in this lake starts around one month before ice-off (between 80 and 110 days since ice formation). The modeled sequence of events during this late winter transition is believed to be as follows: thinning lake ice and melting of the overlying snow, combined with some surface inflows contribute to decreasing conductivity. At the same time, increased light contributes to an increase in autotrophs, reflected in increased chlorophyll and co-occurring increases in diatom counts in late winter. Note, observed data also show green algal counts are constant in winter and make up the largest proportion of the total algal species in winter (Figs. 5-2 & C-4-1). A decline in NH_4^+ is observed, likely resulting from autotrophic uptake (e.g., Chapter 4; Gu and Alexander 1993), with nutrient concentrations also affected by meltwater inputs. SRP also declines around this time, which could be a result of increased autotrophic uptake or concurrent increases in oxygen concentrations and geochemical precipitation or sorption (Boström et al. 1988; Orihel et al. 2017). Expected changes in light would drive increased

productivity, and influxes of oxygen-rich melt water combined with increased productivity would drive changing oxygen concentrations. In fact, the peak in spring bloom is often observed under ice – just prior to ice-off (Fig. 5-3). Modeled negative relationships between nutrients and algal indicators (diatoms and chlorophyll) suggest that biotic uptake in winter may be sufficient to impact nutrient chemistry – helping drive the late winter decline in nutrient concentrations. Although models do not suggest a systematic change in odour during winter, negative relationships between odour and DIN and SRP may similarly reflect changes in the algal community, as odour is associated with increased productivity particularly of green algae (Kehoe et al. 2015).

The late winter conductivity transition point remained consistent in time across this multi-decade time series, when winter conditions varied markedly. This suggests that changes in ice-cover duration (Fang and Stefan 2009) are unlikely to influence when conductivity begins to change, but likely could influence how long the late winter period of declining conductivity lasts. Given the late winter period is characterized by nutrient drawdown, we hypothesize that if this period was to become shorter in a changing climate, increased nutrient availability at ice out could result. One of the effects hypothesized is that the start of seasonal succession of algal species that presently initiates under ice with conditions of lower light, low mixing and abundant nutrients would begin earlier. Under this scenario, there would be greater nutrient availability, light, and mixing potential when earlier ice-out occurs, with the potential for different algal species to dominate (Barica 1990; Pierson et al. 2013; Katz et al. 2015).

However, the key question is whether changing light and temperature during this period would simply allow phytoplankton to drive an earlier nutrient drawdown. Day length is also an important, and rapidly changing parameter in the late-winter period. With climate-change

associated shifts towards earlier ice-out in many regions (Fang and Stefan 2009; Magee and Wu 2017), this suggests interesting changes in the light environment, with lakes becoming ice free when day lengths are shorter. Ultimately the most significant impacts of declining ice cover may be the longer open water season (Bertilsson et al. 2013). It is important to note that although declining ice cover duration has been noted in many regions, there is no evidence of a declining trend in Buffalo Pound Lake (period 1966-2016). The prairies have high natural climatic variability (LaBaugh et al. 2016), which means trends in climate may not be rapidly detectable.

Temperature was not considered in most models, as it covaried strongly with days since ice on. Despite this, we note that thermal changes through winter are important. The temperature ranges seen here, from as low as 0.5°C to as warm as 9.8°C could induce important metabolic changes (e.g., see Welch and Bergmann 1985; Meding and Jackson 2003; Pulkkanen 2013), and control nutrient regeneration while also affecting productivity and nutrient uptake. While much attention has been given to declining periods of ice cover, the extremely variable thermal and light environment through winter deserves further consideration. For example, conditions under ice will be highly sensitive to climate-related changes in wind (altering mixing regime and thus oxygen conditions at fall ice-on as well as snow accumulation on ice), and precipitation (affecting snow), combined with changing frequencies of mid-winter melt (affecting snow, and inputs). Climate change may have equally important impacts on the conditions within winter as it does on the duration of winter ice cover. Understanding both will be important to understanding how changing winters will affect lake ecosystems.

5.6 Conclusions

With rapid declines in the duration of ice cover in temperate lakes, there is strong interest in better understanding lakes during winter, to anticipate future, climate-driven changes. In this shallow, prairie drinking water reservoir, oxygen declined through early winter while conductivity increased. Many other variables were also related to time from ice-on. While strong time dependency is shown, we see many trends reverse themselves in late winter, suggesting understanding future change must be informed by our understanding of key phases of winter, and the physical drivers of these phases. The month before the ice is off is a time of rapid change, with much higher chlorophyll, and oxygen, and lower NH_4^+ and SRP. This period is typified by melting snow and ice, increased inflows, and greater light availability (Chapter 4). Within this lake, no directional trends in ice cover duration are apparent. However, high variability in ice cover duration, and high variability in conditions under ice provide insights into the role of ice cover in structuring lake ecosystems, and the potential impacts of declining ice cover duration. These results suggest that the effects of ice cover duration on algal productivity and nutrient chemistry during the open water season will depend strongly on how the distinct periods of winter change – the duration of the low productivity, high nutrient phase, and the timing and duration of the spring bloom, and nutrient drawdown. While decreases in either phase of winter could impact nutrient concentrations at ice out, the impacts would differ depending on how the early winter nutrient accumulation phase, and the late winter nutrient drawdown phase are affected. Ultimately, these phases may continue to counteract each other. And while a shorter late-winter phase could impact nutrient concentrations at ice-out, the rapidity of changes during the spring bloom suggest it is a key moment, when conditions are re-set. As a result, it may help at least temporarily dampen the

effects of a shorter winters on nutrient chemistry. However, clearly many complex interactions, and effects may occur.

5.7 Acknowledgements

This work would not be possible without the collaboration, support and advice from the staff at the Buffalo Pound Water Treatment Plan, particularly Daniel Conrad, Manager of Laboratory & Research. The authors would like to acknowledge support of NSERC (Discovery Grant to HMB), Environment Canada Science Horizons Program, the Global Institute for Water Security and Canada Excellence Research Chair Funding, the School of Environment and Sustainability, the University of Saskatchewan, and the Teacher-Scholar Doctoral Fellowship for funding this project. For their support and advice, we would like to thank Drs. Rebecca North, Angela Bedard-Haughn, John-Mark Davies, and Cherie Westbrook. Further, we would like to acknowledge the following individuals for their willingness to review statistical methods with an acolyte: Drs. Gavin Simpson, Michael Kehoe, K.P. Chun and Megan Larsen, Jared Wolfe and for those that provided technical and professional assistance: Rosa Brannen, Sherry Olauson, Kate Wilson, Michelle Martel-Andre, Zachary Keesey and all members of the Saskwatche Lab.

5.8 Author contributions

E.C. worked with the data and developed the statistical methods with advice from H.B. Together, they developed the study design. E.C. performed the statistical analyses and wrote the manuscript. H.B. advised at all points during the work and provided edits on the manuscript.

6.0 DISSERTATION CONCLUSION

Here I review contributions of this dissertation in the context of previous research. The last two chapters are presented first, to give context for the discussion of the two process-based chapters. These two chapters (4 & 5) show that not only is winter not static, it consistently has two different key phases. Then I draw in process-related insights (Chapters 2, 3 &4) across my study ecosystems and summarize my findings related to N_2O (Chapters 2, 3 &4). I discuss how this work can help inform our understanding of the impacts of shorter-winter ice cover periods on lake chemistry, and highlight several outstanding research questions related to winter limnology, nitrogen cycling, and the implications of declining periods of ice cover.

6.1 Changes in winter

Winter isolation is characterized by limited hydrologic and atmospheric inputs. Yet, the ice-cover period is not static. Chapters 4 and 5 focused on understanding changes across seasons, and phases within winter. In Chapter 5, I present statistical modeling of water quality data to understand how water quality parameters change during winter in Buffalo Pound Lake. This work reaffirms important conclusions of others (Hampton et al. 2017; Powers et al. 2017a; b; Denfeld et al. 2018) indicating that winter is a unique period for biogeochemical processes. Indeed, it is actually a dynamic period in this shallow prairie lake. Buffalo Pound underwent consistent early winter increases in nutrients and declines in oxygen, trends, which reversed themselves in late winter. The month before the ice is off is a time of rapid change, with indicators of higher productivity (i.e., Chapter 5: in models of chlorophyll, diatom counts), and oxygen, and lower NH_4^+ and SRP. This spring-melt period is typified by melting snow and ice, increased inflows, and greater light availability (Chapter 4 observations; Catalan 1992).

In Chapter 4, I studied shallow prairie wetland ponds. These ponds are numerically abundant across the prairies, dotting large areas of the landscape (Downing et al. 2006; Cheng and Basu 2017). Given the cold climate of the region, they undergo a very long period of ice cover. Some of the very shallow ponds freeze to the bottom. The deeper ponds undergo long periods of anoxia. My work focused on understanding changes in winter through the anoxic period, and spring melt in deeper ponds with a mean depth of 3 m (range 1.3-4.1 m).

Winter anoxia and changes in spring-melt are periods difficult to study, and yet, are critical to understanding how wetland ponds might respond to decreased ice-cover duration. Nutrient accumulation during the winter isolation period was observed. However, with the onset of the

spring-melt period, a decrease in nutrients is seen concurrent with a rapid shift in oxygen and light conditions and as melt water enters these ponds. Nitrogen uptake was a key driver of changes in DIN during the melt period, with maximum NH_4^+ and NO_3^- uptake rates occurring just prior to ice-off, and actually exceeding measurements in the open water season. The influx of oxygen into previously anoxic ponds contributes to a decline in SRP observed over the melt period that may be geochemically driven, with autotrophic uptake also expected to contribute to SRP decline. At the same time these biotic processes are occurring, major hydrological changes are underway. The melt waters are relatively rich in NO_3^- and SRP but lower in NH_4^+. These inputs enter the ponds and are subject to dilution in the larger volume of the wetland ponds. Due to the local hydrology, spring-melt is typically the only time of year when flow could move nutrients out of many pothole ponds (Cade-Menun et al. 2013; Hayashi et al. 2016). My findings suggest that the switch to spring-melt conditions, which typically precede connectivity, will induce a decline in dissolved inorganic nutrient concentrations, naturally protecting downstream ecosystems from high dissolved inorganic nutrient export. However, a proportion of these nutrients may be stored in planktonic algae and dissolved organic matter – and subject to downstream transport ore sedimentation. Due to limitations of this study, I can only hypothesize about the fate of these nutrients. Future work should assess the fate of nutrients assimilated by the spring bloom.

These Chapter 4 results, together with the results of Chapter 5, suggest our understanding of future change must be informed by our understanding of key phases of winter, and the physical drivers of these phases. The effects of declining periods of ice cover on algal productivity and nutrient chemistry during the open water season may depend on how the distinct periods of winter change – the duration of the low productivity, high nutrient phase and the timing and

duration of the spring bloom, and nutrient drawdown phase. While decreases in either phase of winter could impact nutrient concentrations at ice out, the impacts may differ depending on how the early winter nutrient accumulation phase, and the late winter nutrient drawdown phase are affected. Ultimately, these phases may continue to counteract each other. While a shorter late-winter phase (up to four weeks mid-century; Butcher et al. 2015) could impact nutrient concentrations at ice-out, the rapidity of changes during the spring bloom suggest it is a key moment, when biogeochemical conditions are re-set, associated with the rapid physical changes. As a result, it may help at least temporarily to dampen the effects of shorter winters on nutrient chemistry. However, clearly many complex interactions, and effects may occur with key questions remaining regarding the fate and cycling of nutrients assimilated during the spring bloom.

6.2 Winter changes in the nitrogen cycle

While we have a relatively strong understanding of drivers of change to nitrogen concentrations in the open water season, we have a less complete understanding of how chemistry changes through winter, and the key processes driving these changes. Chapters 4 and 5 reveal that there are consistent increases in NH_4^+ over winter while at the same time oxygen and NO_3^- decline. Previous work on changes in winter (Catalan 1992; Gu and Alexander 1993) and process-based observations (Knowles and Lean 1987; Grantz et al. 2014; Soued et al. 2015; Powers et al. 2017a; b) are limited, and focus on nutrient poor ecosystems, and those that remain ice free through winter. My goal was to fill in some of these gaps. Here, I undertook a study of individual processes within the nitrogen cycle, to understand benthic denitrification rates

(Chapter 2), pelagic nitrification rates across lakes (Chapter 3), pelagic nitrogen uptake (Chapter 4) and N_2O in winter (Chapters 2, 3 and 4).

6.3 Denitrification and nitrification

Denitrification is an important mechanism of nitrogen removal (Seitzinger 1988). In chapter 2, I show that denitrification rates were similar across winter and summer, a surprising result when we consider the temperature sensitivity of denitrification rates (Brin et al. 2017), but one which reflects the higher availability of NO_3^- substrates in winter, and evidence of NO_3^--limitation. This finding of relative stability in denitrification rates across seasons is consistent with findings from other winter, but ice-free lakes, including in Lakes Suwa and Elmdale (Hasegawa and Okino 2004; Grantz et al. 2012). This suggests, given the potential for high export of NO_3^- during snowmelt in cold climates (Costa et al. 2017), that water bodies, where present in the landscape, may be able to attenuate NO_3^- loads even during the cold but critical spring-melt period. Adaptation of alternative methods for measuring denitrification, such as MIMS (An and Gardner 2002) for cold temperatures could yield insights into coupled nitrification-denitrification which may be important, particularly in low NO_3^- systems such as these. In addition, future work examining the potential for pelagic denitrification in anoxic water columns would be of value. Certainly further evaluating the importance of denitrification on the larger landscape scale, in winter and spring, could provide improved estimates of NO_3^- removal in cold regions with snowmelt-driven hydrology.

Nitrification is an important nitrogen transformation process, using oxygen and producing NO_3^-, hence producing the substrate for denitrification (Ward et al. 1982). My work in Chapter 3 demonstrates that high pelagic nitrification rates in some prairie lakes can occur and could impact

winter oxygen, particularly when NH_4^+ concentrations are high. I found that the highest nitrification rates were downstream of a wastewater plant and likely influenced by the high NH_4^+ concentrations in the effluent. This impact of nutrient enrichment is consistent with what I found in the ELA: the nitrification rate of an artificially eutrophied lake exceeded (by 6-fold) the nitrification rate of a naturally oligotrophic lake in the same region. These results suggest that nutrient enrichment could contribute to increased nitrification oxygen demand, and increased anoxia risk, although more work across eutrophic and oligotrophic lakes and assessing benthic and pelagic nitrification would help clarify the generality, and magnitude of this impact.

Numerous questions remain regarding nitrification and denitrification in winter. Some center around the importance of mixing – and the question of whether winter-generated NO_3^- from pelagic nitrification is transported to the site of benthic denitrification. Likewise, given low oxygen concentrations affecting many sediments, how important is coupled benthic nitrification-denitrification in lakes with soft organic-rich sediments? Can denitrification occur in a cold anoxic water column – yielding coupled nitrification and denitrification processes in the pelagic zone linked to light availability and mixing? Although NH_4^+ concentrations appear to be an important control on pelagic nitrification rates (Chapter 2; Knowles and Lean 1987; Powers et al. 2017a), there is high variation in relationships between nitrification rates and NH_4^+ concentrations raising important questions regarding the full suite of controls on this process. Ultimately, there are many important questions to explore in these processes which may show high temporal and spatial variability.

6.4 Nitrogen uptake

Algal and bacterial uptake of nitrogen is an important component of the pelagic nitrogen cycle (Kumar et al. 2008). Given the low light conditions during winter and parts of the spring-melt period, understanding nitrogen uptake in winter and spring melt is important to characterizing temporal changes in nitrogen transformation in these periods. Nitrogen uptake during spring-melt was very rapid (maximum observed rate: nitrogen uptake: 50 μg N L^{-1} h^{-1}); much higher than maximum nitrification rates (3.6×10^{-3} μg N L^{-1} h^{-1}). During spring-melt NH_4^+ uptake rates were 50 times higher than the winter rates, and drove the decline in NH_4^+ concentrations observed in spring. While NO_3^- uptake was higher during the melt period, it was still lower than NH_4^+ uptake. This indicates general preferential use of NH_4^+ over NO_3^-. Surprisingly, NO_3^- uptake was higher during the spring-melt period than the open water period just a few days later. The maximum daily pelagic NO_3^- uptake rate, during melt, could amount to~ 56% of the average *in situ* NO_3^- concentrations during the same period – hence uptake is a significant NO_3^- sink. The large amount of uptake and concurrent drawdown of dissolved nutrients that occurs during spring-melt can be attributed to the spring bloom (Gu 2012). The spring bloom is important for productivity, but it appears to be just as important biogeochemically in controlling available nitrogen. Because the spring-melt period is also a time of rapid movement of water, future work should consider the fate of the newly incorporated N. Is that N-rich biomass retained or moved downstream? If it is retained, does it remain near the photic zone and become re-mineralized (or grazed) or does sedimentation play a role in removing it from the photic zone? These are all questions that should be considered to fully assess controls on nutrient export and the forms of nutrients exported.

6.5 The winter nitrous oxide story

Nitrous oxide is 265 times as potent as CO_2 as a greenhouse gas on a 100y timespan (IPCC 2014) and acts as an ozone depleting chemical (Ravishankara et al. 2009). Nitrous oxide is produced as a byproduct of both nitrification and denitrification (Firestone and Davidson 1989), while it is also consumed by denitrification, particularly at low NO_3^- concentrations (Baulch et al. 2011). In this study, I found that lakes, ponds and reservoirs were typically supersaturated with N_2O during the winter and spring-melt periods, which is indicative of active nitrogen cycling during these periods. These findings of frequent winter supersaturation were in contrast to previous work in boreal lakes, which showed substantial under-saturation during winter (Soued et al. 2015). Differences observed here may reflect the higher trophic state of prairie ecosystems. Urban and agricultural nutrient pollution have been linked to enhanced N_2O production (Mosier et al. 1998).

Despite evidence of active nitrogen cycling in winter, winter denitrification rates were not statistically correlated with *in situ* N_2O saturation (Chapter 2). However, higher nitrification rates were strongly associated with higher N_2O saturation (Chapter 3). This could mean that nitrification is the major contributor to N_2O production under ice-cover. While N_2O is generally supersaturated in winter, a few study sites were markedly under-saturated, indicating N_2O consumption. Undersaturation was particularly prevalent later in winter when anoxia may have enhanced consumption of N_2O (Chapter 4) and, as noted elsewhere, N_2O consumption was attributed to denitrification. In the open water period, the majority of measurements show N_2O was undersaturated (Chapter 4). This indicates that, at least during certain times of the year, these water bodies can act as sinks for N_2O. Aquatic N_2O emissions remain poorly characterized (Baulch et al. 2012). These results suggest that N_2O may be highly dynamic in aquatic

ecosystems across seasons, and suggests that additional work is required to better understand how winter nitrogen cycling drives the production and consumption of N_2O and how source and sink strength are affected by the varied conditions of winter.

6.6 Next steps

6.7Light and carbon to nutrient stoichiometry

My work focused largely on nutrients, however there are many interesting areas to explore regarding the intersection of light, carbon and nutrients in the winter and melt periods. Light, as I suggest in Chapters 4 and 5, is a driver of change during winter. Snow cover controls light penetration, playing a crucial role in autotrophic activity and winter mixing (Pernica et al. 2017). Snow cover depends on precipitation, sublimation, fetch (Agbeti and Smol 1995) and the location of the water bodies at low spots or depressions on the landscape (affecting snow redistribution; Pomeroy et al. 1993) hence snow cover, and changing snow cover can be very challenging to predict. Remote sensing of snow cover, and/or continuous light and temperature sensors under ice would be a valuable addition to winter monitoring programs to better understand the dynamic light environment under ice and mixing dynamics, allowing better framing of the physical drivers of changing chemistry

The light-nutrient hypothesis predicts that when light conditions are lower, there will be lower ratios of carbon (C) to nitrogen (N) to phosphorus (P) in particulate matter (Sterner et al. 1997). Photosynthesis, respiration and mineralization processes change these ratios and differences observed over winter could reflect the dominance of those processes (Sterner 2011). But what happens during winter when light conditions are fairly low for long periods? Because the production of new particulate matter slows (low photosynthetic activity), additional work is

required to understand how changes in settling rates, grazing rates, and light interact to influence particulate C:N:P ratios and broader effects on food quality.

The stoichiometry of organic material in winter could have strong impacts on nutrient cycling and the food web (Ferrari 1976; Hampton et al. 2017). Yet, the effects of winter conditions on stoichiometry are relatively understudied. Controls on winter mineralization rates are also inadequately characterized (e.g., within the range of temperature variability observed in lakes under ice cover, and under oxic and anoxic conditions). I have attributed much of the winter changes observed in NH_4^+, and to a degree SRP, to mineralization (Chapters 4 & 5), but direct measurements of mineralization rates, controls on mineralization rates, and the role of internal loading in changing P budgets would provide useful insights. Similarly, we have poor understanding of changes in DOC through winter and melt, or of how winter carbon dynamics might differ among water bodies of different trophic statuses or morphometry or within a water body (e.g., pelagic vs littoral). We do know that DOC can be higher in winter in shallow lakes than the open water period (Hampton et al. 2017), but the trends within winter are largely uncharacterized outside of oligtrophic Lake Redó (Catalan 1992). Significant generation of CO_2 and CH_4 have been observed in winter, yet most studies on these greenhouse gases are in the boreal zone, and lack the influence of agriculture (Denfeld et al. 2018). Further work on winter carbon cycling needs to be done to fill some of these gaps, including how light penetration, landscape, morphology, and trophic status might play a role in carbon dynamics in winter, and how the spring melt period might impact organic carbon via the influx of light, and photochemical reactions.

6.8Winter nitrogen and phosphorus cycle gaps

This work presents denitrification (Chapter 2) and nitrification (Chapter 3) rates across many systems, as well as rates of pelagic NH_4^+ and NO_3^- uptake (Chapter 4). The bulk of this work contrasts rates across seasons, or ecosystems. However, there is a need to better understand spatial variation within an ecosystem (e.g., pelagic, benthic, profundal and littoral areas) and assess how these environments differ in their rates of nitrogen transformation. Because my rates were measured typically at a single point in a given season per study site, including more time points and greater spatial coverage would help build understanding of the extent of variation, and potential for 'hot spots' or 'hot moments' of nitrogen transformation to occur, for example, associated with transient mixing. This intensified approach would also support improved understanding of N_2O dynamics during winter. Recent metagenomic work has revealed how bacterial communities can shift given different environmental conditions (e.g., Wilhelm et al. 2014), and there are strong potential insights from pairing metagenomics work to process-based measurements such as those I have performed here. Monitoring how bacterial communities shift in response to the changing winter conditions could provide a clearer picture of why and how nutrients are changing.

Sulfur is often present in high concentrations in these prairie potholes and could be a player in the nitrogen cycle. It can impact both nitrification (Joye and Hollibaugh 1995) and denitrification (Knowles 1982). Denitrification can increase in the presence of sulfide through sulfur oxidizing bacteria denitrifying NO_3^-. Sulfide can also inhibit the complete conversion of NO_3^- to nitrogen gas, depending on the specific species of denitrifying bacteria (Knowles 1982; Burgin and Hamilton 2007). Nitrification is inhibited in the presence of sulfide (Joye and Hollibaugh 1995) which can be present at the oxic-anoxic interface where coupled nitrification/denitrification may

occur. Understanding the impacts sulfur has on these N mechanisms might provide insight into some of the changes we observe in winter.

While nitrification and denitrification control the availability of NO_3^-, further work could be useful on understanding whether dissimilatory NO_3^- reduction to NH_4^+ (DNRA) is an important process in these ecosystems, and winter controls on the process. Because DNRA occurs under anaerobic conditions, and leads to NH_4^+ generation, it could be interesting to assess rates of DNRA in systems such as the prairie pothole ponds studied in Chapter 4 (Burgin and Hamilton 2007). These sulfur-rich ponds could be important spots of DNRA because of the associated sulfur reduction by some chemolithoautotrophs (Burgin and Hamilton 2007). Recent advances in measurement of DNRA and denitrification rates on the membrane inlet mass spectrometer (An and Gardner 2002; Roland et al. 2017) could be used to understand these processes in controlling both NH_4^+ and NO_3^-.

The majority of my dissertation work has focused on winter nitrogen cycling, however, P dynamics are just as interesting. My work in Chapters 4 and 5 show that SRP tends to increase at the beginning of winter and decline towards the end. Previous work in winter has shown that sediment P release rates in winter are often lower than the open water period (6 of 8 sites had lower winter rates of P release; Orihel et al. 2017). However, local work in the prairies (Lake Diefenbaker) suggests that P release can be even higher than the open-water season (North et al. 2015), while work on Buffalo Pound lake suggests lower P release in winter (D'Silva 2017). Low oxygen conditions could aid release of P from sediments through the dissolution of P from iron oxides (North et al. 2015). Given that specific geochemistry varies spatially and during winter sediments may experience oxic or anoxic conditions (Mathias and Barica 1980), winter SRP release may be highly variable as a result.

Phosphorus loading from the sediments during winter could explain the initial winter increases in SRP observed in Chapters 4 and 5. Low P uptake in the early winter, I would predict, could allow for the accumulation of P, similar to what I found for NH_4^+ (Chapter 4). However, there are few measurements of P uptake in winter or under low light conditions. Likewise pelagic P uptake was not measured during the spring-melt period when SRP concentrations declined (Chapters 4 & 5). Without process-based measurements, the fate of this P is unclear, and future work to understand the controls on sediment P release under ice, and the relative importance of P loss during spring melt to biotic P uptake vs geochemical sinks would be beneficial. Finally, the rate at which P assimilated in spring is lost to sedimentation, recycled from sediment, or recycled from biomass is not known. Clearly, more detailed study of the influence of spring melt conditions on P availability during the open water period would be beneficial.

6.9 Climate change

Many temperate water bodies are ice-covered for much of the year. As the climate warms, the duration of ice-cover will continue to decrease (Fang and Stefan 2009). There is clear evidence ice cover duration is shortening (Vincent et al. 2015), that there is earlier winter spring-melt (Bonsal et al. 2006) and that streams begin to flow earlier (Kam et al. 2018). However our understanding of what these changes means to water quality is limited (Anteau et al. 2016), and in many ways, constrained by gaps in what we know about winter limnology under current conditions. Climate change poses risks to water bodies through the predicted increase in inflow of nutrients (Blenckner et al. 2007), worsening issues of eutrophication, and increased risk harmful cyanobacterial blooms (Paerl and Huisman 2008). Further, climate change is predicted to change seasonal precipitation patterns. Winter snows are likely decreasing in the Prairies (Shook

and Pomeroy 2012) causing winter light conditions to change. Less snow in winter could increase light penetration and, potentially increase under ice productivity, although altered mixing dynamics may not mean net increases in light to a phytoplankton cell (Pernica et al. 2017). Further work needs to be done to understand some of these complex winter processes, as I have outlined in the above sections, in order to move towards understanding potential climate change impacts on our water resources associated with the shortened duration of winter and help understand priorities for moving forward.

Modeling is a tool at our disposal that can help us understand how future change might impact lake conditions. Despite some of the gaps filled by this dissertation, there is much more work required to effectively model winter biogeochemistry, and inform scenarios of declining ice cover duration. Likely one of the main drivers of change during winter is light – light penetration can increase water temperatures, induce mixing (Pernica et al. 2017), enhance photosynthesis (Catalan 1992), and likely change nutrient dynamics. Yet, light records under ice are rare, and intermittent – poorly matched to the dynamic environment that may exist under ice. Work with satellite imagery, combined with advances in sensor technology may help us better understand links between changes in the physical, chemical and biotic components of lakes through winter.

Ultimately, lakes have undergone and will continue to undergo many changes, associated with exposure to many stressors. As we discuss declining periods of ice cover and the implications for hydrochemistry for both catchments and lentic ecosystems, it is imperative that we understand the mechanisms that drive changing conditions through winter, and understand the individual phases of winter. The study of aquatic ecosystems under the ice has been relatively limited until recently (but see Schindler et al. 1974; Catalan 1992; Gu and Alexander 1993). My work seeks to fill some of the gaps in our understanding of nitrogen cycling in winter (Chapters

2, 3 & 4) and within winter shifts in oxygen, nutrients and primary producers (Chapters 4 & 5). These chapters demonstrate that in winter, lentic ecosystems undergo many changes, with surprisingly dynamic nutrient cycling under ice. Additional integrative work is required to characterize the impacts of decreased ice cover duration on nutrient cycling and primary productivity, and understand how these changes will interact with climate change and other stressors during the open water season. More sustainable management of water resources will require a holistic understanding of changes across seasons, and in space – to help build an understanding of how future changes will impact key ecosystem services.

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Appendix A. Supplemental Information Chapter 2: Denitrification under Lake Ice

The supplemental information contains three tables with further information about the study sites (Table A.1), temperature sensitivity of denitrification for our study sites and winter and summer comparison of rates in other cold aquatic ecosystems (Table A.2) and denitrification rates from the literature using similar methods as a comparison with this work (Table A.3). Details of sampling design, analyses, and study sites are in the main text (Chapter 2).

Table A.1 Study sites and corresponding sampling information, day of year median denitrification rates and corresponding *in situ* data. Ice-on typically occurs in November or December for these study systems.

Location	Date	Day of Year	Mean depth	Season	Surface area	Denitrification Rate	Nitrate Amended Denitrification Rate	Oxygen	Specific Conductance	pH	Temperature	Nitrate	Ammonium	Nitrous oxide
			(m)		(ha)	($\mu g\ N\ g^{-1}h^{-1}$)		($mg\ L^{-1}$)	($\mu S\ cm^{-1}$)		(°C)	($\mu g\ N\ L^{-1}$)		(% Saturation)
Blackstrap Reservoir	6-Mar-14	65	5	ice-cover	1200	1.48×10^{-5}	6.89×10^{-2}	2.2	1137	7.6	4.2	75.9	167	165.3
Blackstrap Reservoir	18-Jul-14	199	5	open-water	1200	2.80×10^{-4}	1.39	10	1540	8.5	21.1	40†	86†	80.4
Broderick Reservoir	19-Mar-14	78	6	ice-cover	376	ND***	4.1×10^{-1}	0.1	637	7.3	4.3	259	39.6	78.4
Broderick Reservoir	29-Jul-14	210	6	open-water	376	7.91×10^{-4}	3.12	8.7	372	8.1	19	21.2	22.9	83.4
Buffalo Pound Lake	11-Mar-14	70	3	ice-cover	2910	5.05×10^{-4}	5.32×10^{-3}	0.7	856	7.4	4.2	318.6	117.3	235
Buffalo Pound Lake	20-Jun-14	171	3	open-water	2910	8.61×10^{-4}	1.68	NA	NA	NA	NA	40†	86†	NA
Echo Lake*	23-Feb-16	54	10.2	ice-cover	1300	1.22×10^{-3}	6.00	6.7	1751	8.5	2	176.8	493	NA
Echo Lake*	18-Aug-16	231	10.2	open-water	1300	9.92×10^{-3}	1.9×10^{-1}	3.7	1712	8.6	20.6	14.8	NA	NA
Katepwa Lake**	10-Mar-15	69	14.3	ice-cover	1620	6.66×10^{-2}	2.7×10^{-1}	9.5	1555	6.7	0.9	615.4	86†	299.5
Katepwa Lake**	21-Aug-15	233	14.3	open-water	1620	2.67×10^{-4}	1.94	0	1312	7.7	11.1	40†	987.5	NA
Lenore Lake, South Basin	25-Mar-15	84	5.8	ice-cover	1450	7.41×10^{-4}	1.7×10^{-1}	1.2	3810	7.5	2.2	244.2	327	285.5
Lenore Lake, South Basin	26-Aug-15	238	5.8	open-water	1450	1.96×10^{-3}	3.08	7.5	3040	8.7	17.4	40†	86†	75.5
Pasqua Lake	10-Mar-15	69	6.5	ice-cover	1790	8.69×10^{-4}	1.07	5.2	2185	7	1	531.8	2250	692.9
Pasqua Lake	21-Aug-15	233	6.5	open-water	1790	1.86×10^{-4}	5.7×10^{-1}	9.1	1600	8.8	21	50.9	86†	NA
St. Brieux Lake	25-Mar-15	84	6.6	ice-cover	221	2.79×10^{-3}	1.54×10^{-2}	0	3664	6.1	1.1	578.5	86†	455
St. Brieux Lake	26-Aug-15	238	6.6	open-water	221	2.64×10^{-3}	6.8×10^{-1}	0.1	3018	7.9	15.7	40†	176	77.4
St. Denis Pond 1	8-Apr-14	98	1.3	ice-cover	9	1.59×10^{-3}	1.7×10^{-1}	0.1	5072	7.2	1.3	40†	1701.5	27.3
St. Denis Pond 1	16-Jul-14	197	1.3	open-water	9	6.76×10^{-5}	6.8×10^{-1}	0.1	4008	8	19	40†	453.6	18.4

*Lake water chemistry was taken at 0.5 m above the sediment with the exception of Echo Lake where water samples were obtained from 1 m above the sediment. **Katepwa Lake sediments were obtained from near shore (2 m water depth), with water chemistry samples from 0.5m above the sediment. ***ND: No data

†Indicates that sample concentrations were below method detection limits of 86 and 4 $\mu g\ N\ L^{-1}$ for NH_4^+ and NO_3^-, respectively. NA indicates data were not reported.

Table A.2 Denitrification rates and temperature sensitivity of rates in cold or ice-covered aquatic ecosystems. Q10 is the ratio of one higher temperature rate to one lower temperature rate corrected for the temperature difference scaled to 10°C*. Q10 gives an indication of the increase in rate as the temperature increases. Important to note for this study is the dramatic increase in this ratio when NO_3^- is added.

Aquatic Ecosystem	Season	Ice-Covered	System Name	Year	Method of analysis	Treatment (if applicable)	Measurement site	Denitrification Rate	Units	Ratio of Winter to Summer Rates	Q10	Data source
Reservoirs, lakes, ponds	Summer		Saskatchewan	2014-2016	Acetylene inhibition	Ambient Nitrate	Sediment	$7.91x10^{-4}$**	$\mu g\ N\ g^{-1}h^{-1}$	1.32	1.02*	This Study
Reservoirs, lakes, ponds	Winter	Yes	Saskatchewan	2014-2016	Acetylene inhibition	Ambient Nitrate	Sediment	$1.04x10^{-3}$**	$\mu g\ N\ g^{-1}h^{-1}$		2.7*	This Study
Reservoirs, lakes, ponds	Summer		Saskatchewan	2014-2016	Acetylene inhibition	Amended Nitrate	Sediment	1.39**	$\mu g\ N\ g^{-1}h^{-1}$	0.12	2.3*	This Study
Reservoirs, lakes, ponds	Winter	Yes	Saskatchewan	2014-2016	Acetylene inhibition	Amended Nitrate	Sediment	0.17**	$\mu g\ N\ g^{-1}h^{-1}$		5.7*	This Study
Lake	Summer		Lake Suwa, Japan	1994	Acetylene inhibition		Sediment core	94.04	$\mu mol\ N\ m^{-2}h^{-1}$	7.46		Hasegawa and Okino 2004
Lake	Winter	No	Lake Suwa, Japan	1994	Acetylene inhibition		Sediment core	701.35	$\mu mol\ N\ m^{-2}h^{-1}$			Hasegawa and Okino 2004
Bay	Summer		Ariake Bay, Japan	2006-2008	Acetylene inhibition		Sediment core	533.12	$\mu mol\ N\ m^{-2}d^{-1}$	0.22		Koriyama et al. 2016
Bay	Winter	No	Ariake Bay, Japan	2006-2008	Acetylene inhibition		Sediment core	115.54	$\mu mol\ N\ m^{-2}d^{-1}$			Koriyama et al. 2016
Stream	Summer		Walker Branch and Noland Creek, Tennessee	1998-1999	Acetylene inhibition		Sediment	535.95	$ng\ N_2O\ g^{-1}h^{-1}$	0.33	2.49	Martin et al. 2001
Stream	Winter	No	Walker Branch and Noland Creek, Tennessee	1998-1999	Acetylene inhibition		Sediment	175.05	$ng\ N_2O\ g^{-1}h^{-1}$		1.29	Martin et al. 2001
Lake	Winter	Yes	Lake Nästjärn	2014	Acetylene inhibition	15°C	Sediment core	23	$\mu mol\ N_2O\ m^{-2}h^{-1}$	0.61		Myrstener et al. 2016
Lake	Winter	Yes	Lake Nästjärn	2014	Acetylene inhibition	4°C	Sediment core	14	$\mu mol\ N_2O\ m^{-2}h^{-1}$			Myrstener et al. 2016
Lake	Winter	Yes	Lake Nästjärn	2014	Acetylene inhibition	Ambient Nitrate	Sediment core	0.66	$\mu mol\ N_2O\ m^{-2}h^{-1}$		1.69	Myrstener et al. 2016
Lake	Winter	Yes	Lake Nästjärn	2014	Acetylene inhibition	Amended Nitrate	Sediment core	10	$\mu mol\ N_2O\ m^{-2}h^{-1}$			Myrstener et al. 2016
Lake	Permanent Ice Cover	Yes	Lake Bonney, Antarctica	1999-2000	Acetylene inhibition		Water	0.733	$nM\ N_2O\ h^{-1}$		2.41	Ward et al. 2005

*Q10 was calculated via: $(R2/R1)^{(10/T2-T1)}$ (Brown & Sibly, 2012), ** Median rates are reported for this study, other rates are pulled from the literature text, charts or figures.

Table A.3 Denitrification rates (μg N g-1h-1) for this study (median rates for 9 reservoirs, lakes and ponds) and literature rates of similar (or easily converted) units and using the acetylene inhibition method.

Aquatic Ecosystem	Season	System Name	Year	Treatment (if applicable)	Measurement site	Denitrification Rate	Data source
Reservoirs, lakes, ponds	Summer	Saskatchewan	2014-2016	Ambient Nitrate	Sediment	0.0008	This Study
Reservoirs, lakes, ponds	Winter	Saskatchewan	2014-2016	Ambient Nitrate	Sediment	0.001	This Study
Reservoirs, lakes, ponds	Summer	Saskatchewan	2014-2016	Amended Nitrate	Sediment	1.389	This Study
Reservoirs, lakes, ponds	Winter	Saskatchewan	2014-2016	Amended Nitrate	Sediment	0.172	This Study
Reservoirs	May	Tobacco Creek, Manitoba	2013		Sediment	0.17	Gooding 2015
Reservoirs	June	Tobacco Creek, Manitoba	2013		Sediment	0.46	Gooding 2015
Reservoirs	July	Tobacco Creek, Manitoba	2013		Sediment	0.0032	Gooding 2015
Reservoirs	August	Tobacco Creek, Manitoba	2013		Sediment	0.0006	Gooding 2015
Streams	May	Tobacco Creek, Manitoba	2013		Sediment	0.0019	Gooding 2015
Streams	June	Tobacco Creek, Manitoba	2013		Sediment	0.0003	Gooding 2015
Streams	July	Tobacco Creek, Manitoba	2013		Sediment	0.0023	Gooding 2015
Streams	August	Tobacco Creek, Manitoba	2013		Sediment	0.0019	Gooding 2015
Streams	Summer	Kalamazoo River Catchment			Sediment	0.5 - 1	Arango and Tank 2008
Streams	Winter	Kalamazoo River Catchment			Sediment	1.5 - 3	Arango and Tank 2008
Soils		Iowa		Ambient Temperature	Soils	118 - 446	Breitenbeck and Bremner 1987
Soils		Iowa		4°C	Soils	118 - 453	Breitenbeck and Bremner 1987
Soils		Iowa		-4°C	Soils	227 - 867	Breitenbeck and Bremner 1987
Stream	Autumn	Walker Branch and Noland Creek, Tennessee	1998-1999		Sediment	0.0121 - 1.004	Martin et al. 2001
Stream	Winter	Walker Branch and Noland Creek, Tennessee	1998-1999		Sediment	0.0501 - 0.3	Martin et al. 2001
Stream	Spring	Walker Branch and Noland Creek, Tennessee	1998-1999		Sediment	0.152 - 1.231	Martin et al. 2001
Stream	Summer	Walker Branch and Noland Creek, Tennessee	1998-1999		Sediment	0.0309 - 1.041	Martin et al. 2001
Soils					Soils	$1.8 \times 10^{-7} – 2.0 \times 10^{-5}$	Smith and Tiedje 1979

Appendix B. Supplemental Information Chapter 4: The rise and fall of nutrients in ice covered ponds

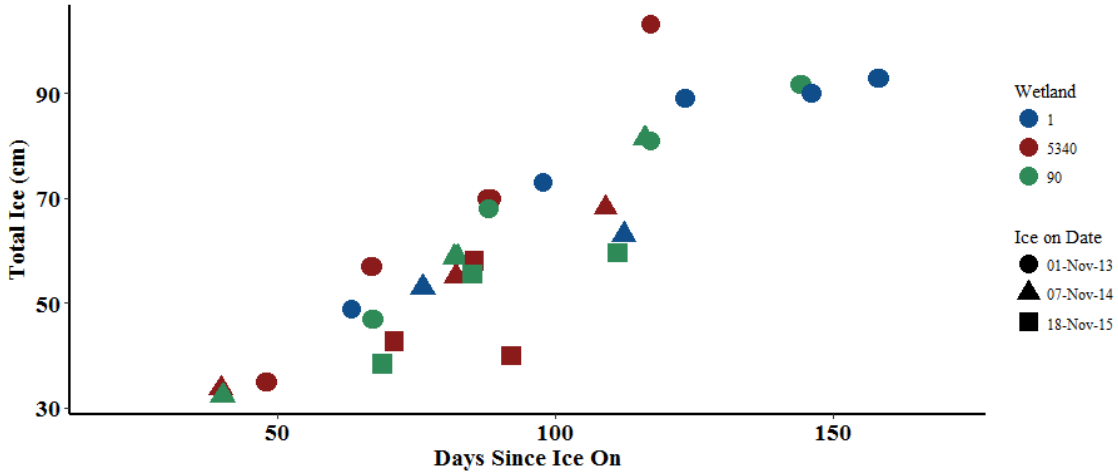

Figure B.1 Total ice thickness as measured in the field over the course of three years at three different wetland ponds.

Appendix C. Supplemental Information Chapter 5: Winter Matters

Table C.1 Nitrogen uptake measured in Buffalo Pound on February 9, 2016. Methods for the uptake experiment follow Chapter 4, Section 4.3.3, following the methods outlined by (Gu et al. 1997). Sample water (collected from the raw intake from the BPWTP) was incubated under low temperature (4°C) and light conditions (~30 µmol m-2s-1; simulating light conditions under ice-cover) and dark conditions. All measurements were above the limits of quantitation as calculated as per Chapter 4, Section 4.3.3.

Treatment	Ammonium Uptake ($\mu g\ N\ h^{-1}\ L^{-1}$)	Nitrate Uptake ($\mu g\ N\ h^{-1}\ L^{-1}$)	Chlorophyll a ($\mu g\ L^{-1}$)
Light & 4-hour Incubation	2.11×10^{-3}	1.65×10^{-1}	19
Dark & 4-hour Incubation	1.66×10^{-3}	1.24×10^{-1}	19

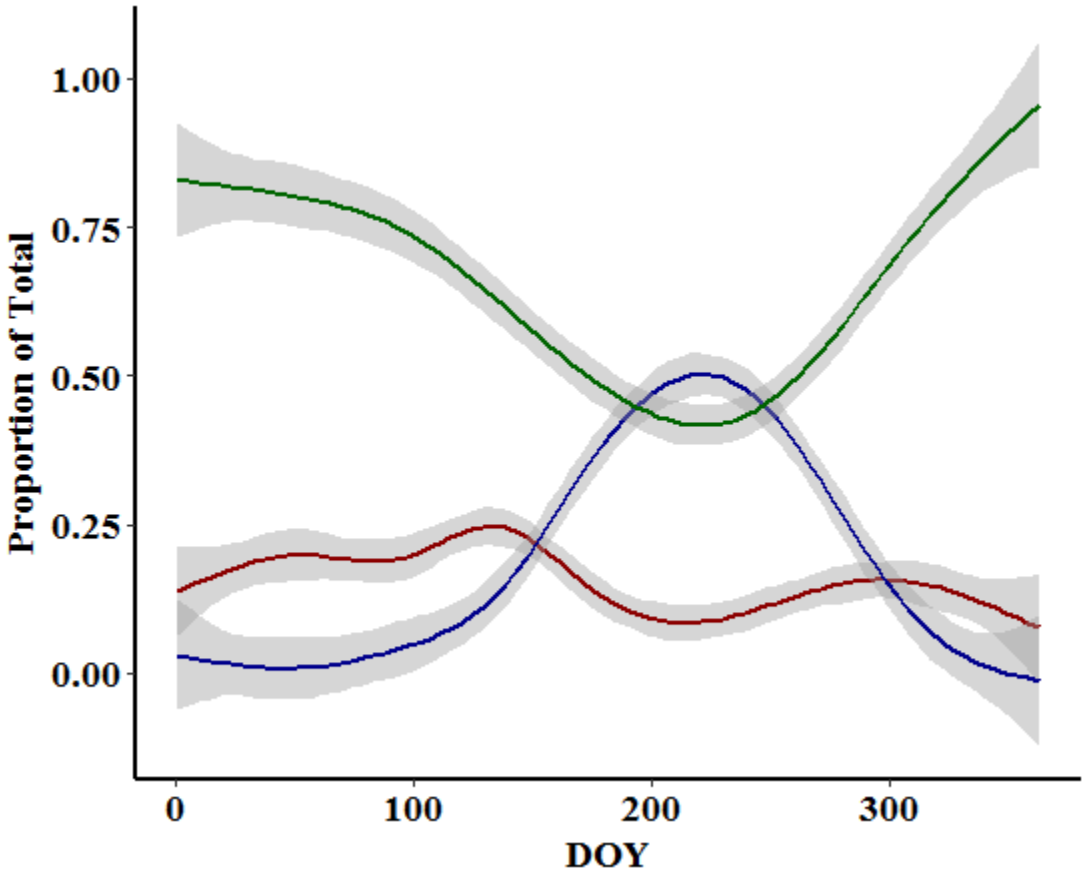

Figure C.1 The lines represents the loess fit of the proportion of diatoms (red), green algae (green) and blue-green algae (blue) as a fraction of the total of the same three species.

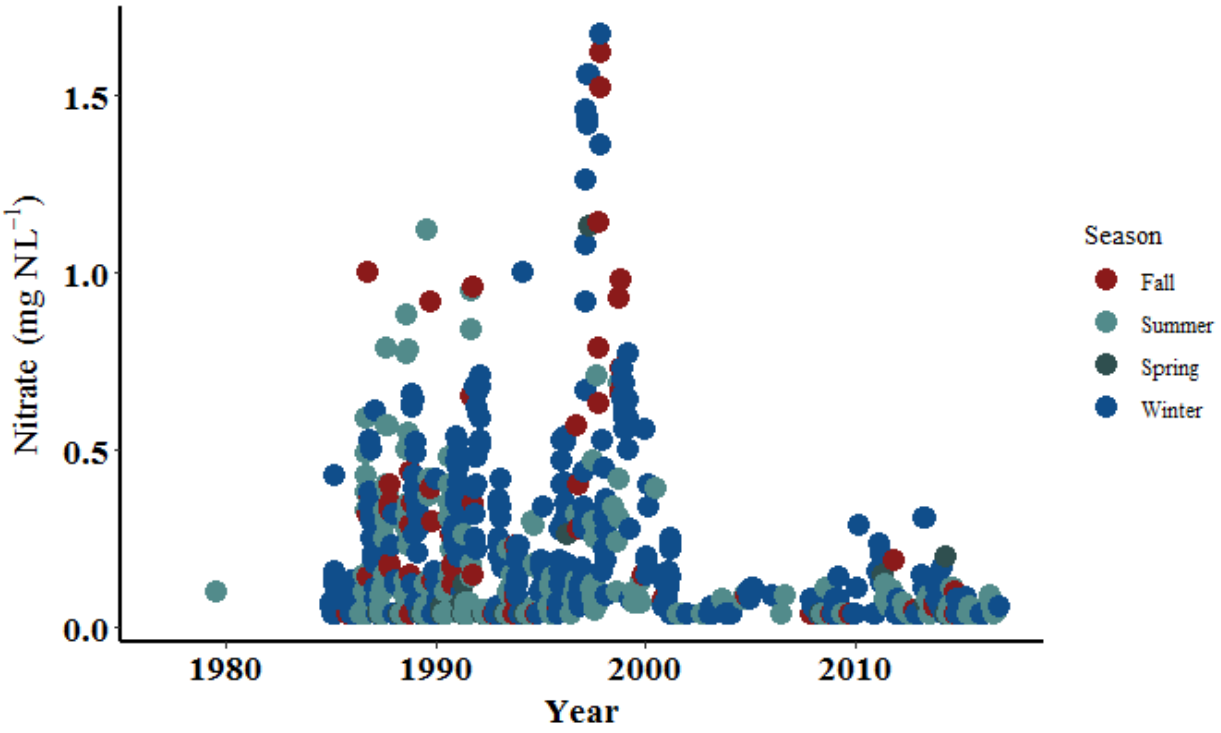

Figure C.1 Nitrate concentrations over the time series in Buffalo Pound. Dramatic decrease in concentrations began around 2000 – with significant decline noted.